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PHYTOPATHOLOGY

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THE FIRST SYMPTOM OF TOMATO FUSARIUM WILT: CLEARING OF THE ULTIMATE VEINLETS IN THE LEAF

R. E. FOSTER[1]

(Accepted for publication March 13, 1946)

The later symptoms of typical Fusarium wilt of the tomato, caused by *Fusarium oxysporum* f. *lycopersici* (Sacc.) S. & H., have been described by many workers since the disease was first seen prior to 1895 (1, 4). The symptoms most commonly mentioned have been stunting, progressive yellowing and wilting of the leaves, wilting and collapse of the stem, and death of the plant, all accompanied by an increased darkening of the vascular elements. Wellman (5) recently described epinasty as an early symptom of tomato wilt. A still earlier symptom of this disease has been seen repeatedly, and it is believed to be the first above-ground indication of infection of the tomato by the wilt pathogen.

This early symptom is a clearing of the ultimate veinlets in the leaflets of infected tomatoes giving them a "netted" appearance (Fig. 1). It can be seen only when leaves are viewed with transmitted light. In a detailed study of 50 young tomato plants (4 to 6 leaves) inoculated with a virulent strain of the organism and held at optimum conditions for wilt development (optimum moisture, 28° C.) and observed at 12-hour intervals, it was noticed that veinlet clearing appeared on many plants 24 hours after dip-inoculation. Most often it was first evident in the terminal leaflet of the third leaf, after 36 hours appearing in the second and fourth leaves and later in all of the leaves. Veinlets closest to the main veins became cleared first and the large veins themselves seemed to be outlined by a very narrow, cleared band. Occasionally, this clearing progressed more rapidly on one side of the leaflet. The loss of green color soon progressed along all veinlets and later extended into the "vein-islets" which, just previous to veinlet clearing, appeared to be a darker green. As the clearing spread from the veinlets, yellowed areas were formed in the leaf which later coalesced, giving rise to the "yellow-leaf" symptom of the disease. Forty-eight hours after dip-inoculation veinlet clearing was present in all leaves. Unilateral inoculation of tomato plants with the fungus occasioned a unilateral development of this early symptom. Epinasty did not appear in any of the plants until 72 hours after inoculation, and in most it was not evident until after 84 hours. Veinlet clearing seems to be associated entirely with the effects of *Fusarium oxysporum* f. *lycopersici* on the tomato host. Noninoculated plants held under the same conditions did not have the veinlet clearing symptom. Repeated attempts to recover the organism from affected areas in the leaf 36 hours after inoculation were unsuccessful. Likewise, attempts

[1] The writer wishes to express his gratitude to Eugene Herrling for the preparation of the illustration. Investigation supported in part by a grant from the Wisconsin Alumni Research Foundation.

FIG. 1. Portions of terminal leaflets from (A) a healthy tomato plant, and (B) an inoculated tomato plant: ×4.

to produce the symptom on healthy tomato and tobacco leaves by rubbing them with juice expressed from affected leaflets met with no success. Symptoms identical to those described have not been seen in association with any other tomato diseases, although it is recognized that other vascular parasites might produce toxic materials which could bring about a similar effect.

Clearing of the ultimate leaf veinlets of inoculated tomato plants has been observed in several host varieties and under various environal conditions. Bonny Best, Marglobe, Master Marglobe, and Rutgers (*Lycopersicon esculentum* Mill.) varieties all showed the early symptom. However, the time interval between inoculation and first appearance of veinlet clearing varied with the relative resistance of the variety. It has not been found in a highly resistant strain of Red Currant tomato (*L. pimpinellifolium* Mill.) or in highly resistant hybrids. In inoculated susceptible plants grown under a wide range of environal conditions (3), the production of the veinlet-clearing symptom was not affected by soil moisture, soil temperature, air temperature, light conditions, or host nutrition, except in relation to time of appearance, as long as the particular environment permitted an ultimate development of tomato Fusarium wilt. The three strains of *F. oxysporum* f. *lycopersici* tested brought about development of the early symptom. These three strains were (a) virulent, raised strain R 5–6, (b) mild, appressed strain A 15–8, both obtained from F. L. Wellman (6), and (c) a strain of intermediate pathogenicity from J. B. Kendrick, California. The rapidity with which the netting symptom became evident varied with the pathogenicity of the fungus strain.

Numerous attempts were made to produce the early, veinlet-clearing symptom in young plants placed in fungus extracts in a manner similar to that used by Fisher (2). Young Bonny Best plants (5 to 6 leaves) were grown in soil, were dug, the roots were cut off under water, and then the stems were placed in filter-sterilized Richard's nutrient in which the virulent strains of the *Fusarium* had been growing for different lengths of time. Veinlet-clearing symptoms appeared only in plants placed in the medium from an agitated 28-hour culture. Media from older cultures produced yellowing and necrosis rapidly, without signs of veinlet clearing; younger cultures were not used. Water and Richard's solution controls produced no symptoms.

Very early symptoms of tomato Fusarium wilt are of little importance in studying the disease as it occurs in the field, but such symptoms have been of considerable value in various controlled studies in the greenhouse and laboratory. Moreover, the rapid appearance of evidence of host-parasite interaction suggests a phase of disease development that has received little consideration. It is widely accepted that the disease effects brought about by vascular fusarial infection are occasioned largely by toxin produced in the infected root and spread throughout the plant in the transpiration stream. The appearance of a disease-induced effect in the upper portion of a plant only 24 hours after root inoculation indicates that toxin

is produced at the time of fungus penetration or very shortly thereafter, and certainly before the organism is well established within the host tissue. Whether the toxin causing veinlet clearing is produced by the fungus or by the plant in response to fungus action has not been shown definitely. However, the former possibility seems the more likely in view of the fact that the same peculiar symptom can be induced by aseptic fungus extract. The observations presented seem to contribute to the theory that the disease reactions brought about by vascular Fusaria are conditioned by the formation of more than one type of toxin depending upon the degree of parasite invasion and consequent host reaction.

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BACTERIAL LEAF SPOT AND BUD ROT OF ORCHIDS CAUSED BY PHYTOMONAS CATTLEYAE

PETER A. ARK AND H. EARL THOMAS

(Accepted for publication April 26, 1946)

A large number of orchid-growing establishments in California often sustain heavy losses from brown-spot disease in such valuable species as *Cattleya* and *Phalaenopsis*. Microscopic examination of diseased specimens has always revealed the abundance of bacteria in advancing margins of the spots. In order to ascertain the causal agent of the disease a bacteriological study of the isolates was made. This paper presents the results of the investigation as to the nature of the disease, the causal organism, and the method of control.

Elliott (1) lists the following organisms as causal agents of disease in orchids: *Bacillus cypripedii, Bacillus farnetianus, Bacillus pollacii, Bacterium cattleyae, Bacterium krameriani,* and *Bacterium oncidii.* These organisms attack orchid leaves and pseudo-bulbs, causing occasional death of the plants. Recently (3) a soft rot of *Cattleya sp.,* caused by *Erwinia carotovora,* was reported in the United States.

Erwinia carotovora was also reported as a pathogen on *Phalaenopsis aphrodite* in Japan (4) and *Cymbidium aloifolium* and *C. insigne* were successfully inoculated. The disease starts as water-soaked spots on the leaf blades, which become sunken and brown. The organisms enter the plant through wounds. Matsumoto and Okabe (4) presented evidence which indicates that the *Bacillus cypripedii* of Hori (2) is very closely related to, if not identical with, *E. carotovora.*

For a number of years the writers have studied a disease of orchids which occurs in *Phalaenopsis sp.* and *Cattleya sp.* and sometimes causes considerable damage in orchid houses in the San Francisco Bay region. The disease is prevalent in greenhouses in which a high air humidity is maintained for considerable time and where the plants are syringed. A bacterial organism was isolated from the advancing margins of the lesions, proved pathogenic upon inoculation, and was readily reisolated.

At first the disease expresses itself as small, dark, water-soaked spots, which rapidly increase in size, changing from light-brown to dark chestnut brown with age. These spots upon enlarging may coalesce to form larger areas (Fig. 1, A and B). Under favorable conditions of temperature and moisture the infection spreads rapidly and may attack the crown, sometimes killing the plant. The disease may be initiated through wounds, but the organism can apparently cause infection by direct penetration of uninjured leaves.

Phalaenopsis sp. and *Cattleya sp.* were infected under conditions which approximated those obtaining in commercial greenhouses. The following orchids were susceptible when artificially inoculated: *Epidendrum o'brein-*

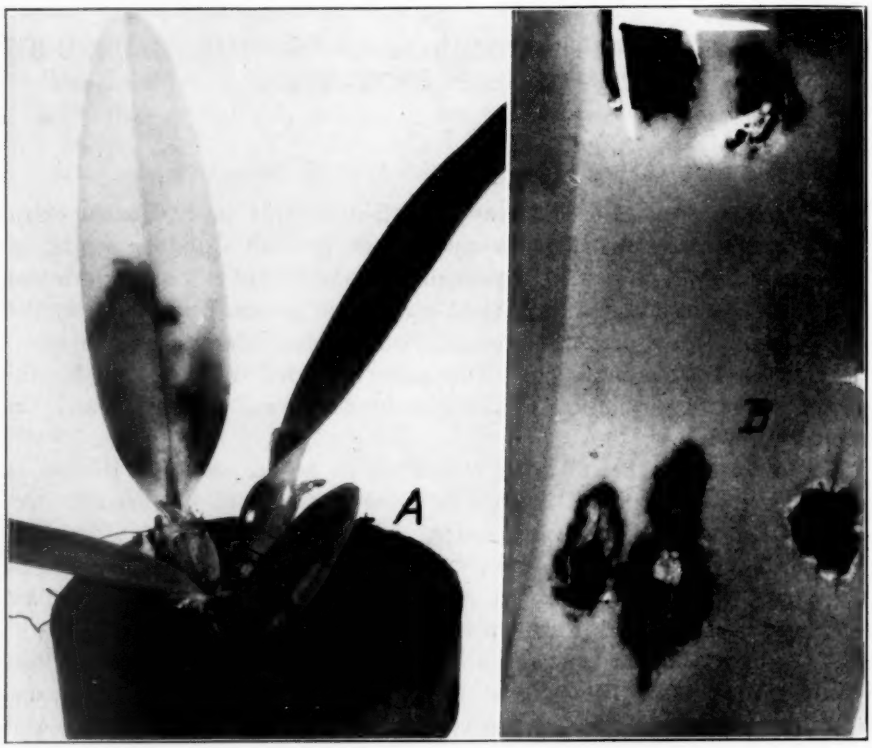

FIG. 1. Bacterial leaf spot on *Cattleya sp.* A. Natural infection. B. Detail of a well-advanced sunken spot on ventral side of a leaf.

ianum, Dendrobium sp., Cypripedium sp., Phalaenopsis amabilis and Vanilla. No infection could be obtained on *Phalaenopsis luedemanniana.*

Four isolates of the organism, together with four reisolates, were studied bacteriologically in order to ascertain the taxonomic position of the causal agent. All cultures behaved identically in the laboratory and had certain features ascribed to *Bacillus farnetianus, Bacillus pollacii, Bacterium cattleyae,* and *Bacterium krameriani.* However, as Elliott (1) pointed out, there is no certainty that the work on these four species was done with pure cultures. All were imperfectly described, so that with few exceptions they would be interchangeable (Table 1).

TABLE 1.—*Comparison of cultures causing orchid leaf spots according to Pavarino (5)*

Bacterium cattleyae	*Bacillus farnetianus*	*Bacterium krameriani*	*Bacillus pollacii*
2.4μ × 0.4–0.6μ	1.5μ × 0.8–1.0μ	2–3μ × 0.6–0.8μ spore-forming	8–10μ × 1.0μ
Gram-negative	Gram-positive	Gram-negative	Gram-negative
White on agar	Iridescent to grayish on agar	Greenish on agar	Greenish on agar
Gelatin not liquefied	Gelatin liquefied	Gelatin liquefied	Gelatin liquefied

A description of the organism causing the disease occurring in California is given below. This bacterium is probably the one which was described by Pavarino (5) as *Bacterium* (*Phytomonas*) *cattleyae,* since all the characters of this organism, as indicated by Pavarino, including the symptoms of the disease, are in close agreement.

Phytomonas cattleyae. Small, Gram-negative rods in stained smears of 24-hour-old beef-extract-peptone-agar growth, single and in pairs; motile by means of one or two lophotrichous flagellae; no spores as determined by staining and by heating to 60° C. for 20 minutes.

On beef-extract-peptone agar of pH 6.9, at 28° C., growth is rapid, grayish-white with iridescence when examined by transmitted light. The consisteney is butyrous. The colonies are large, entire, smooth, with criss-cross markings resembling fish scales. On potato-dextrose-peptone agar slants the growth is very rapid and dirty-gray. On potato plugs the growth is rapid and slimy, dark-gray. Beef-extract-peptone broth becomes very turbid within 24 hours with the formation of a very delicate pellicle which later drops to the bottom, leaving a rim of growth on the walls of a test tube. Good growth occurred in liquid synthetic media such as: synthetic carbohydrate media of the Society of American Bacteriologists (6, p. 15), Fermi, Cohn, and Uschinsky. Starch is digested slowly. Gelatin is not liquefied even after thirty days. Hydrogen sulphide and indol are not produced. Nitrates are reduced to nitrites. Both skimmed and litmus milk were unchanged when the cultures were observed 3, 7, and 14 days after inoculation. Ammonia is produced in nutrient broth after 3 days, determined by the method of Hansen (7, p. 15). Acid and no gas is produced in a synthetic carbohydrate medium of the Society of American Bacteriologists (6, p. 15) to which one per cent of arabinose, dextrose, dulcitol, galactose, glycerol, lactose, levulose, mannite, sucrose, or xylose was added and as an indicator 0.5 cc. of a one per cent alcoholic solution of brom cresol purple and 0.4 cc. of a one per cent solution of cresol red to each liter of the medium and a Dunham fermentation vial (inverted) to each tube to detect gas. Neither acid nor gas was formed from raffinose. The optimum temperature for growth lies betwen 25° and 35° C. The thermal death point is 48° C.

To obtain satisfactory control of the disease, badly diseased plants should be removed and individual spots on the leaves treated by carefully swabbing with a sponge soaked in 1 to 1000 corrosive sublimate ($HgCl_2$). No injury of the treated leaves was observed, either on *Cattleya* or *Phalaenopsis.* A considerable reduction in the disease was obtained when the greenhouse humidity was decreased and overhead irrigation discontinued.

SUMMARY

1. A bacterial leaf spot and bud rot of *Cattleya sp.* and *Phalaenopsis sp.* caused considerable damage in orchid houses in Central California.

2. The disease may be controlled by reducing the air humidity and by swabbing diseased plant parts with 1 to 1000 corrosive sublimate solution.

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SEEDLING DISEASE OF YELLOW CALLA, CAUSED BY CORTICIUM SOLANI, AND ITS CONTROL

C. M. Tompkins and P. A. Ark

(Accepted for publication May 23, 1946)

INTRODUCTION

A seedling disease of the yellow calla (*Zantedeschia elliottiana* Engler) has long been prevalent in field-planted seedbeds in and near Santa Cruz, California. Annual losses ranged from 25 per cent or more, with occasional complete failures. The results of field and laboratory studies of this disease during the past 5 years, with suggestions for control, are presented in this paper.

SYMPTOMS OF THE DISEASE

Yellow calla seedlings may become infected before or after emergence. The symptoms of the disease on the roots of infected plants consist of a dry, brownish-black discoloration of the cortical tissues, generally commencing at the tip of the roots and progressing toward the crown. Sometimes infection occurs above the tips of the roots. The cortex of infected roots soon shrivels and sloughs off, exposing the central cylinder. Seedlings infected before emergence are small and die very quickly. This phase of the disease can only be observed by digging into the soil just before the seedlings emerge, which usually occurs in from 4 to 6 weeks after sowing the seed. The post-emergence phase of the disease is equally destructive. Apparently healthy seedlings with 2 to 6 leaves may suddenly turn yellow, wilt, and collapse. Death ensues in a day or two and may affect single seedlings or a group of them in the same row. When removing diseased seedlings from the soil, little or no pressure is required. A third and equally important phase of the disease is rotting of the seed before germination; this is also an important factor in the reduction of stand. Rotting of the seed and seedling infection before and after emergence can usually be observed in any seedbed. However, seasonal variations, coupled with unfavorable cultural practices, may accentuate the loss from seed decay or, on the other hand, seedling mortality (pre- or post-emergence) may be the most conspicuous feature of the disease.

This disease occurs on widely different soil types, ranging from sandy and gravelly loams to heavy clay, and is favored by excessive irrigation, poor drainage, warm weather, improperly prepared seed, mulching, and crowding of the plants.

THE CAUSAL FUNGUS

Diseased yellow calla seedlings, showing different stages of root infection, were collected at Capitola and Santa Cruz each summer from 1941 to 1945, inclusive, for cultural tests in the laboratory. After washing the roots in tap, distilled, and sterile distilled water, followed by drying on clean filter paper, small bits of root tissue were planted on potato-dextrose agar in

Petri dishes. Isolations each year consistently yielded but 1 fungus which has been identified as the vegetative stage of *Corticium solani* (Prill. and Del.) Bourd. and Galz. Isolates were obtained from hyphal tips of the fungus in Petri-dish cultures. Comparison of the isolates obtained each season from different seedbeds showed a remarkable similarity in macroscopic characters.

The relation of temperature to growth of the mycelium was determined for 2 isolates of *Corticium solani*, 1 from Capitola and 1 from Santa Cruz. The culture tubes (2.1 by 20 cm.) used and the procedure followed were those previously described by Tompkins and Gardner.[1] The medium used was potato-dextrose agar, pH 5.8. Inoculated tubes were kept at room temperature for 24 hours. Then 3 tubes of each isolate were placed in a horizontal position in controlled temperature chambers at intervals of 3°, from 4° to 40° C. The cultures were incubated for 96 hours. The cardinal temperatures were determined on the extent of mycelial growth in the culture tubes.

The minimum temperature for growth of the yellow-calla isolates of *Corticium solani* was approximately 7° C., the optimum 25° to 28°, and the maximum 37°.

These isolates were also used in the infection experiments in the greenhouse. Inoculum was prepared by growing the isolates on sterilized, moistened cracked wheat in 8-inch test tubes. When ready for use, the inoculum of either isolate was added to each of 6 flats of steam-sterilized, sandy-loam soil. After the soil and inoculum had been thoroughly mixed, the flats were set aside for 1 week. Simultaneously, controls were prepared by adding the same amount of sterilized cracked wheat to each of 4 flats of steam-sterilized soil. The soil in all flats was stirred and irrigated on alternate days before seeding. Each flat was planted at a depth of 1 inch with 500 untreated yellow calla seeds, obtained at Santa Cruz, at intervals of ½ inch in the row, the rows being 1 inch apart. Prevailing greenhouse temperatures ranged from 14° to 18° C. After 5 weeks, seedlings appeared above ground in all flats. Disease was prevalent in all infested flats, as indicated by the yellow foliage, collapse of the seedlings, and skips in the row. In general, the symptoms on artificially-infected plants were identical with those of naturally-infected plants. Infection counts were made from the fifth to the ninth week, inclusive. The Capitola isolate caused a pre-emergence loss of 25 per cent and a post-emergence loss of 48 per cent, or a total of 73 per cent. The Santa Cruz isolate caused a pre-emergence loss of 32 per cent and a post-emergence loss of 51 per cent, or a total of 83 per cent. All yellow calla seedlings in the control flats remained healthy for the duration of the tests. The fungus was reisolated from 50 infected plants in each of the infested flats; the reisolates were identical with the original isolates. When reisolates of the Santa Cruz and Capitola isolates were tested, they proved highly pathogenic.

[1] Tompkins, C. M., and M. W. Gardner. Relation of temperature to infection of bean and cowpea seedlings by *Rhizoctonia bataticola*. Hilgardia **9**: 219–230. 1935.

CONTROL OF THE DISEASE

Since field studies at Capitola and Santa Cruz suggested that certain cultural methods and lack of seed treatment were important factors related to the high incidence of infection in yellow calla seedbeds, control was predicated on improving the cultural conditions in the beds and on treatment of the seeds with a suitable fungicide.

Control experiments were conducted at Santa Cruz for 3 years. Briefly, the method of control was based on (1) thorough cleaning of the seed; (2) dusting the seed with an equal mixture of Spergon (tetrachloro para-benzoquinone) and Celite 505 (an infusorial earth used as a carrier); (3) wider spacing of seeds to prevent subsequent crowding of seedlings in the beds; (4) avoidance of excessive irrigation; (5) frequent cultivation of the soil to provide good aeration; (6) selection of a well-drained site; and (7) elimination of organic soil mulches.

Limited field tests, based on the foregoing procedure, were conducted in 1942, resulting in almost perfect stands of seedlings. In the control seedbeds, handled according to prevailing commercial practice, seedling disease was as destructive as in previous years. Because of the ease of applying Spergon anl its effectiveness, no other fungicidal dusts were tested. In 1944 and 1945, the field tests were enlarged because more seed was available. Results were comparable with those obtained in 1942. The adoption of this method for controlling the disease in yellow-calla seedbeds has been recommended to the growers.

DISCUSSION

Improper processing of dried, yellow-calla spadices, in the fleshy pulp of which the seeds are imbedded, yields unclean seeds, *i.e.*, the seeds are partially or entirely covered with a layer of dried pulp. After these seeds are planted and the beds irrigated, the pulp attached to the seedcoats readily absorbs moisture and conceivably becomes a moist chamber surrounding each individual seed. Thus the way is prepared for infection by the pathogen. To circumvent this condition, it is suggested that the spadices be crushed and washed in tap water. Then the viable seeds, which sink to the bottom of the container, should be washed at least 4 times, dried on clean absorbent paper, and dusted with a fungicide, such as Spergon.

Close planting of yellow calla seeds, long an established commercial practice, insures crowding and predisposes the young seedlings to heavy infection. It would be desirable to plant the seeds 1 to 2 inches apart in short rows, the depth varying from 1½ to 2 inches, with rows 6 inches apart.

Daily irrigation of seedbeds is another common practice which favors the disease. Semi-weekly or weekly irrigation of the seedbeds would provide ample moisture for germination and growth and would aid in decreasing the amount of infection. The selection of a well-drained site is of paramount importance.

Some growers cover their seedbeds to a depth of 1 or more inches with

organic materials, such as macerated coffee husks, in order to conserve the moisture. Mulches not only interfere with good soil aeration, but conceivably they serve as moist chambers for fungus invasion. Their use should be avoided.

In late summer, in mature yellow calla plantings, the withering peduncles are unable to support the weight of the spadices, which lodge on the ground. Collections of spadices were made for 3 years in order to test the pulp and seeds in the laboratory for the presence of *Corticium solani*. The fungus was never isolated from these plant parts.

SUMMARY

A destructive seedling disease of yellow calla (*Zantedeschia elliottiana*) is prevalent in commercial plantings in the Capitola–Santa Cruz section of California. It is favored in its development and spread by warm weather, excessive moisture, poor soil drainage, the use of unclean, untreated seed, close planting of seed, and mulching.

The principal symptoms of the disease consist of a brownish-black discoloration of the cortical tissues of the fibrous roots (before and after emergence), usually commencing at the tips and progressing toward the crown. Invaded tissues shrivel and slough off. Above ground, infected seedlings develop yellow leaves which wilt, collapse, and die. Rotting of the seed may also occur before germination.

Corticium solani (Prill. and Del.) Bourd. and Galz. has been consistently isolated from infected seedlings and has proved pathogenic in greenhouse tests.

The minimum temperature for mycelial growth of the 2 isolates studied is 7° C., the optimum between 25° and 28°, and the maximum 37°.

The disease can be readily controlled by using clean seed treated with a suitable fungicide, such as Spergon, planting the seed at wider intervals in the seedbeds, avoiding excessive irrigation and mulching, selecting a well-drained site, and cultivating frequently to provide good soil aeration.

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THE EFFECTS OF CERTAIN NUTRIENT TREATMENTS UPON THE RESISTANCE OF COTTON TO FUSARIUM VASINFECTUM[1]

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INTRODUCTION

The resistance of certain crop plants to various pathogenic soil organisms can be affected by subjecting the host plants to nutritional conditions which influence the physiological responses of the plants. In a recent monograph, Garrett (3) has reviewed various aspects of this question in relation to root disease fungi. Wingard (14) has reviewed comprehensively the literature on general disease resistance in plants; and, more recently, Shear and Wingard (5) have suggested that the greater susceptibility of sweet corn to *Phytomonas stewartii* when grown under conditions of inadequate potassium nutrition may be due to an accumulation in the conducting tissues of nitrates which are favorable for growth of the parasitic bacteria and unfavorable for rapid growth of the corn plant under conditions of potassium deficiency.

The literature on the influence of nutrition on the wilt resistance of cotton has been reviewed by Young (15), who found that applications of potassium fertilizers to Arkansas soils for the correction of potassium deficiency was accompanied by marked reduction in wilt severity. His work was confirmed by Dick and Tisdale (2) in Alabama, who also reported that under some conditions nitrogen fertilizer applications alone could effectively reduce wilt severity but that wilt severity was increased following applications of phosphatic fertilizers. Young and Tharp (16) reported increased wilt severity in Arkansas soils following nitrogenous and phosphatic fertilizers, particularly if the fertilizers were not adequately balanced with respect to potassium. A. L. Smith (6) summarized results of a regional wilt test in 9 states and reported reduced wilt following applications of potassium fertilizers, but stated that varietal resistance to wilt was more important than fertilization so far as yield was concerned. Some of the variations in results were attributed to a combination of wilt and nematode infestation in the field plots observed. In a survey of Texas soils, Taubenhaus and coworkers (8) found a correlation between the presence of *Fusarium* in soils and their acidity, with *Fusarium* more prevalent in acid soils.

In sand culture studies Neal (4) reported that wilt resistance of cotton was not increased when plants received a supplementary solution containing

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[2] The author wishes to extend to Dr. G. M. Armstrong and Dr. H. D. Barker his sincere thanks for their cooperation during the progress of these experiments and in the preparation of this manuscript.

calcium nitrate as the source of nitrogen. The basic nutrient solution contained nitrate and ammonium nitrogen in a 7 : 1 ratio. More recently Tharp and Wadleigh (9, 10) studying wilt resistance of several cotton varieties grown in sand cultures in relation to sources of nitrogen and to relative acidity found a significant interaction between nitrogen source and high pH. There was an increase of wilt resistance at pH 8 with nitrates as the source of nitrogen and a decrease in resistance with ammonia as the source of nitrogen at the same pH, with varietal differences in response. Increases in nitrogen and phosphorus levels resulted in decreased wilt resistance while increases in the potassium level of the solutions increased wilt resistance. Armstrong and Schappelle[3] noted that cotton plants grown in a solution in which all the nitrogen was supplied as calcium nitrate were attacked less severely by wilt than plants grown in solutions containing nitrogen from calcium nitrate and ammonium sulphate.

In 1943 and 1944 experiments were conducted by the writer to study in greater detail the responses of a highly wilt-susceptible variety of cotton, Half and Half, to different sources of nitrogen in nutrient solutions at two levels of pH.

MATERIALS AND EXPERIMENTAL METHOD

Pyrex glass jars, of 4½ gal. capacity, 12 in. in diameter and 12 in. in height were coated on the outside with black enamel to exclude light and buried to within a few inches of the top in the soil of the greenhouse benches. Covers were of glazed stoneware or of "masonite hardboard" with 5 two-inch-spaced holes in each cover. Four-hole, flat, split corks were used for supporting the plants in these jars. Sixteen plants were mounted in four corks in each jar and the remaining opening in the jar cover was used for the insertion of a glass tube 25 mm. in diameter for draining and refilling the jars and for a smaller glass tube (6–9 mm. in diameter) through which air for the aeration of the solutions was conducted. Changing of solutions could be accomplished in a few minutes with a minimum of handling and disturbance of plant roots.

Solutions were drained and replaced with new solutions at weekly intervals except at the beginning of the experiments when the plants were small. In 1943 cultures in Series 1 and 2 were aerated several hours daily by bubbling air through them with the aid of a small blower and in 1944 aeration of those of Series 3 was continuous, utilizing air from a storage tank and compressor. Water was added to the solutions from time to time to replace transpiration losses and to avoid excessive changes in concentration of salts in the solutions other than those due to absorption of nutrients by the plants.

Sulphuric-acid-delinted seed dusted with Spergon (tetrachloro para-benzoquinone) were planted in trays of steamed river sand. After 3–4 days the seedlings, selected for uniformity, were transferred to the corks in

[3] Unpublished results from the South Carolina Agricultural Experiment Station, 1937.

the jars of nutrient solutions. Growth of seedlings was rapid and in 12–14 days they were at an advanced seedling stage, but squares had not yet appeared. Inoculation with the wilt fungus was made at that time.

The cultures of *Fusarium vasinfectum* used for inoculation were grown according to the technique used by Armstrong (1). Inoculation was accomplished by a 10-minute immersion of the seedling plant roots in a mycelial suspension of the fungus, after which the plants, together with mycelia adhering to the roots, were replaced in their respective nutrient solutions.

There were three jars of each solution and the location of these was randomized so as to permit subsequent statistical analysis. Non-inoculated or check cultures were also grown for each solution.

The molar concentrations of the salts used are presented in table 1. Stock solutions of convenient concentration of all the nutrient salts were prepared and presented no problems, except in the case of calcium sulphate. Stock solutions of this salt, because of its low solubility, had to be prepared in dilute concentration in considerable volume. In mixing the solutions the monohydrogen potassium phosphate was always added last, with stirring, to the full volume of solution; but even with this precaution there was turbidity in the solutions of higher pH, indicating a certain amount of precipitation of the salts. Except for sulphur the molar concentrations of all nutrient elements were the same in the solutions used. Iron was supplied as ferric citrate in a concentration of 2 p.p.m. of ferric iron. Manganese was supplied in a concentration of 1 p.p.m. and copper, zinc and boron in concentrations of 0.5 p.p.m. for each element.

The height of the plants in each jar was measured at the time of inoculation and twice weekly thereafter. After inoculation the plants were inspected daily for the appearance of wilt symptoms. After these had appeared inspections were made twice weekly and the wilt index of the plants was recorded. Normal plants, mildly wilted, moderately wilted, severely wilted, and very severely wilted or dead plants were given values of 0, 1, 2, 3, 4, respectively, which were used to compute the wilt indices. Since 16 plants were grown in each solution culture the greatest possible wilt index for any one solution was 16×4, or 64. This wilt index was necessarily of an arbitrary nature but it enabled the development of wilt in each jar to be readily followed. Dead plants or plants given a value of 4 were removed from the jars and a portion of the base of the stem cultured in the laboratory to recover the wilt fungus.

Two levels of pH were used in the solutions because of the differences in absorption of ammonium and nitrate ions by plants at different pH levels reported by Tiedjens and Robbins (12). The high pH solutions had an initial pH of 6.6–6.8 and the low pH solutions a pH of 5.0–5.3. In Series 1 and 2 (1943) changes in solution pH were measured from time to time but no attempt was made to maintain the solutions at their initial pH. In Series 3 (1944) an attempt was made to correct wide changes in pH due to

TABLE 1.—*The molar concentrations and pH of the solutions used*

Salt	Low pH			High pH		
	Calcium nitrate nitrogen	Ammonium sulphate nitrogen	Ammonium nitrate nitrogen	Calcium nitrate nitrogen	Ammonium sulphate nitrogen	Ammonium nitrate nitrogen
$MgSO_4 \cdot 7H_2O$	0.002	0.002	0.002	0.002	0.002	0.002
K_2SO_4	0.00075	0.00075	0.00075	.....	.....	.....
KH_2PO_4	0.0025	0.0025	0.0025	0.001	0.001	0.001
K_2HPO_4	.....	.....	.....	0.0015	0.0015	0.0015
$(NH_4)_2SO_4$	.....	0.002	.....	.....	0.002	.....
$Ca(NO_3)_2 \cdot 4H_2O$	0.002	.....	.....	0.002	.....	.....
NH_4NO_3	.....	.....	0.002	.....	.....	0.002
$CaSO_4 \cdot 2H_2O$	.....	0.002	0.002	.....	0.002	0.002
Fe, Cu, Mn, Zn, Bo	Trace	Trace	Trace	Trace	Trace	Trace
Initial pH (Series 1, 2, and 3)	5.0	5.3	5.3	6.7	6.8	6.8
Approximate[a] pH (Series 1 and 2) at time of solution change	6.3	3.9	5.2	6.9	4.3	5.5
Approximate[a] pH (Series 3) at time of solution change	5.1	5.2	5.5	6.8	6.0	6.1

[a] These pH values are approximations derived from the pH measurements and are indicative of the pH trends observed in these solutions. Close control of the pH was not obtained except for the high pH nitrate solutions.

absorption of nutrients by the plants by the addition of either a dilute sulphuric acid solution or a suspension of calcium hydroxide.

PRESENTATION OF DATA

Table 1 shows the molar concentration of the salts used and the initial pH of each solution. The two lower lines of figures show the trend of pH changes in the solutions of all series. The high pH calcium nitrate solutions did not vary appreciably from their initial pH at any time, although the pH increased slightly from 6.6–6.8 to 6.8–7.0. In Series 1 and 2 where no attempt was made to compensate for changes in solution acidity due to differential absorption of nutrients by the plants the low pH calcium nitrate solutions became less acid. In Series 3 the addition of small amounts of

TABLE 2.—*The number of plants grown in each treatment and the number of plants that escaped infection*

Treatment	Series 1		Series 2		Series 3	
	Total No. of plants	No. of plants not infected	Total No. of plants	No. of plants not infected	Total No. of plants	No. of plants not infected
Low pH $(NH_4)_2SO_4$	48	2	47	0	48	0
High pH "	43	0	48	14	42	0
Low pH NH_4NO_3	47	0	48	2	46	0
High pH "	48	6	47	5	48	1
Low pH $Ca(NO_3)_2$	48	8	48	2	48	0
High pH "	47	27	45	11	48	17

dilute sulphuric acid to these solutions kept them near the initial and desired pH. In Series 1 and 2, both high and low pH ammonium sulphate solutions increased in acidity to the point where the growth of the cotton plants was markedly retarded. In Series 3, with excess acidity offset through the use of a calcium hydroxide suspension added from time to time to the solutions, a considerably better growth of plants resulted, although in general vigor and appearance these plants were inferior to those grown

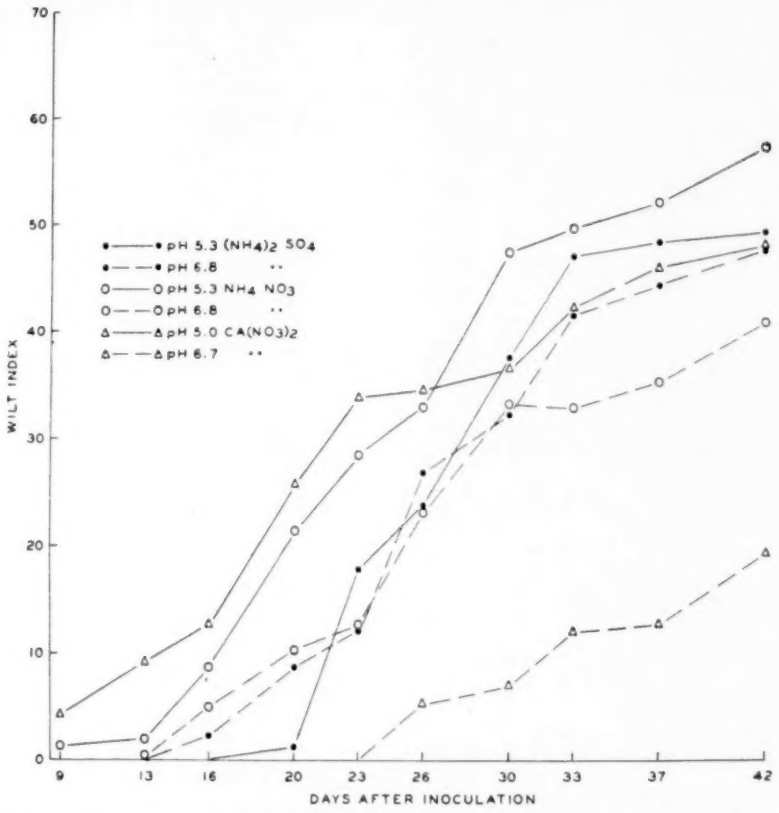

FIG. 1. The mean sum of the wilt indices of the plants in each solution of Series 1 on the indicated number of days after inoculation. Inoculated July 15, 1943.

in calcium nitrate and ammonium nitrate solutions. The pH changes of the ammonium nitrate solutions were intermediate between those of the calcium nitrate and ammonium sulphate solutions. In Series 1 and 2 the high pH ammonium sulphate and ammonium nitrate solutions became more acid and the attempt to correct this acidity in Series 3 was not entirely successful. It should be pointed out that the changes in pH of the solutions presented in table 1, because of the logarithmic character of pH values, are approximations useful in indicating trends rather than representing true means.

The largest and most vigorous noninfected or healthy plants were pro-

duced in the low pH calcium nitrate solutions. Slightly less favorable conditions for growth were present in the high pH calcium nitrate solution and in the ammonium nitrate solutions of both high and low pH. In the ammonium sulphate solutions of Series 1 and 2, in which the pH was 3.8 and 4.5, plant growth was poor, but in Series 3, where excessive acidity was corrected, growth was improved, although markedly less than in the calcium nitrate and ammonium nitrate solutions.

The wilt index values and the percentages of severely wilted or dead

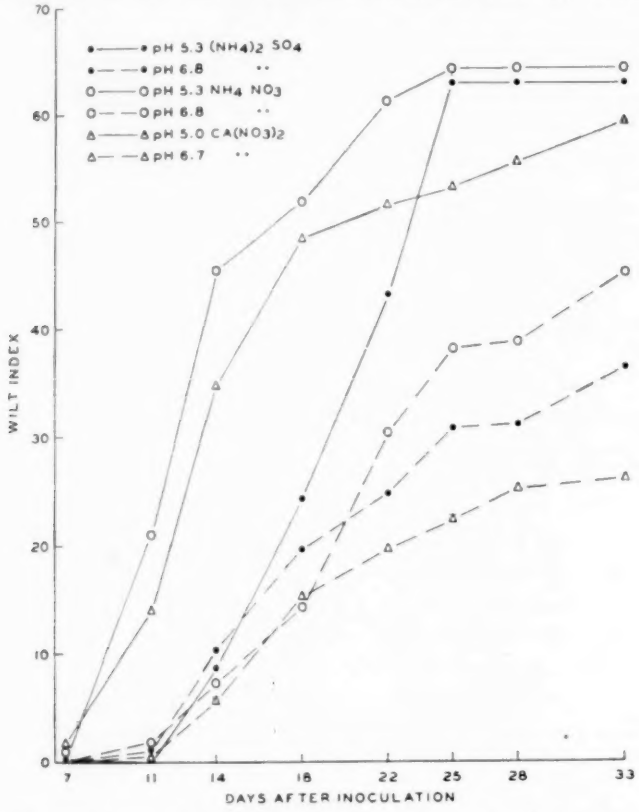

FIG. 2. The mean sum of the wilt indices of the plants in each solution of Series 2 on the indicated number of days after inoculation. Inoculated Sept. 24, 1943.

plants are shown in figures 1, 2, and 3, and figures 4, 5, 6, respectively. Table 2 shows the number of plants of each treatment which had no visible wilt symptoms and from which the *Fusarium* was not recovered on plate cultures at the end of the experiment. The number of plants grown in each solution is also shown in table 2. A considerable number of plants in each series of the high pH calcium nitrate solutions and in the high pH ammonium sulphate solutions of Series 2 apparently did not become infected after exposure to the fungus. The considerable number of noninfected plants in the high pH calcium nitrate solutions of all series indicated that

plants in such solutions were not so readily infected as in other solutions. Some of the plants in the high pH calcium nitrate solutions of Series 3 had blackened vascular tissue although the plants had no wilt symptoms nor could the organism be recovered from plate cultures at the end of the experiment, suggesting that initial infection may have occurred but that the *Fusarium* had not survived. The absence of infection of plants in the high pH ammonium sulphate solutions of Series 2 was probably due to chance since it occurred only in this instance.

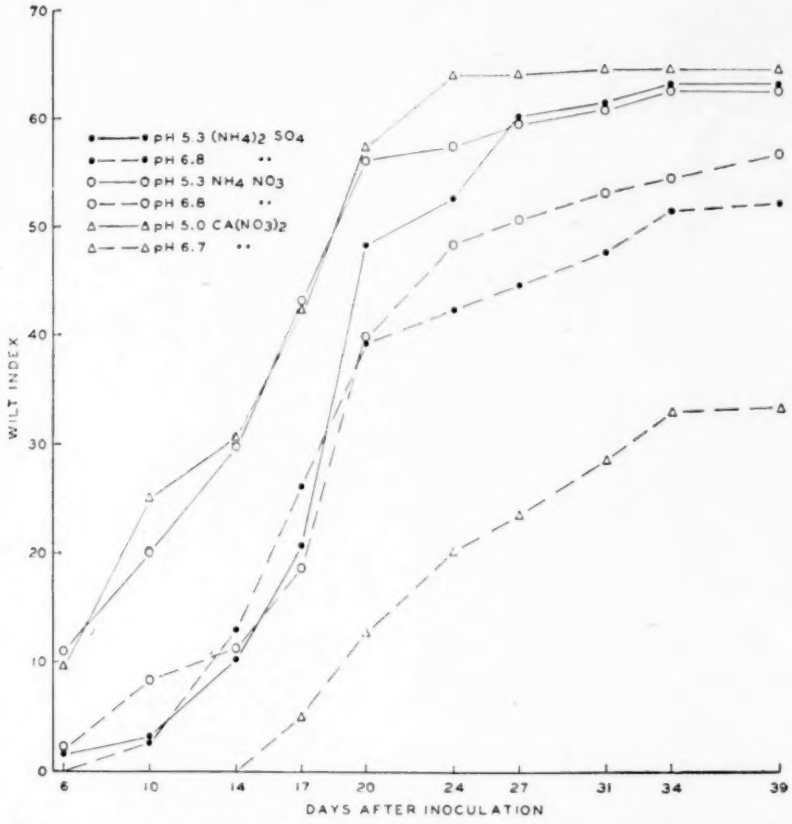

FIG. 3. The mean sum of the wilt indices of the plants in each solution of Series 3 on the indicated number of days after inoculation. Inoculated Aug. 26, 1944.

The single degree of freedom method of analysis described by Snedecor (7) and also discussed by Wadleigh and Tharp (13) and by Tharp, Wadleigh, and Barker (11) in their nutritional studies of cotton, was followed in the statistical comparisons of the data presented in figures 1–6. The general methods followed are illustrated in table 3. The results of individual comparisons are shown in table 4.

DISCUSSION

In general, wilt symptoms began appearing 5–7 days after inoculation and appeared first in low pH calcium nitrate and low pH ammonium nitrate

solutions. In these solutions plant symptoms appeared earlier and wilt development was more rapid than in the ammonium sulphate solutions, but after plants in the latter solutions began to show symptoms of wilt they usually deteriorated quickly. As is shown in figures 1, 2, and 3, the plants in low pH solutions had more pronounced wilt symptoms early in the experiment than plants in high pH solutions, but in time these differences became less, with the exception of the high pH calcium nitrate solutions,

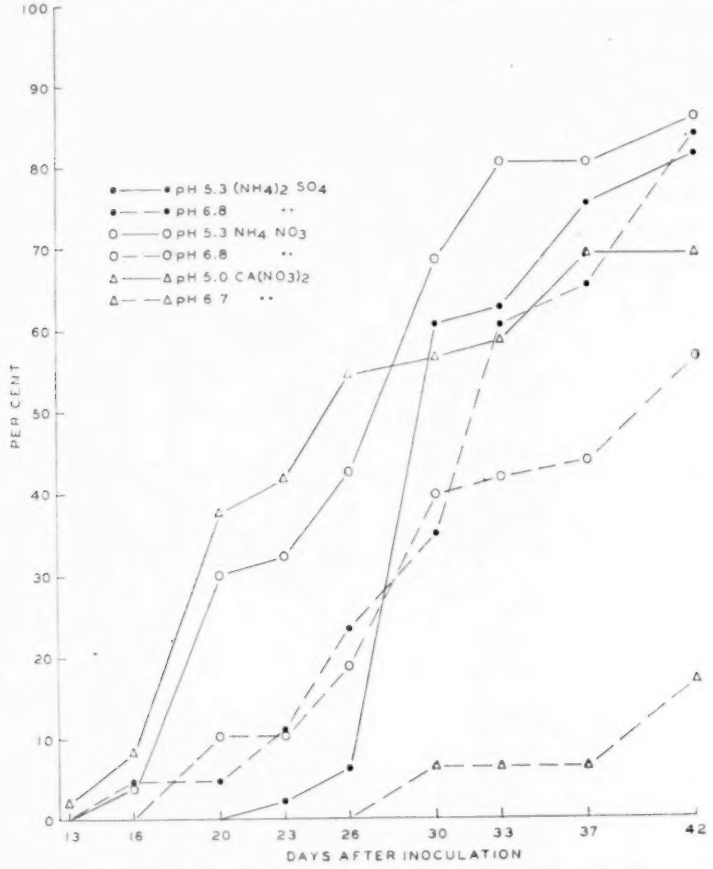

FIG. 4. The percentage of severely wilted and dead plants in cultures of Series 1 on the indicated number of days after inoculation. Inoculated July 15, 1943.

where the appearance of symptoms was delayed and the wilt severity was significantly reduced.

Inspection of figures 1–6 shows that each series of plants, necessarily grown under varying seasonal conditions, showed varying degrees of wilting. The differences between comparisons of treatments in Series 1, as shown in table 4, were less pronounced than those for Series 2 and 3. This is reflected in the smaller number of statistically significant differences in Series 1. The trend, however, was the same as shown by the plus (+) and minus (–) values for each comparison.

Table 4, comparison 1, shows that the plants grown in low pH solutions had a higher wilt index and a higher number of severely wilted and dead plants than the plants grown in high pH solutions, except for the data of August 7. Comparisons 2, 3, and 4 show several differences of probable significance, but they are isolated instances, and do not indicate a consistent trend in the 3 series of plants, and will not be further discussed. Plants grown in low pH calcium nitrate solutions as compared with those grown

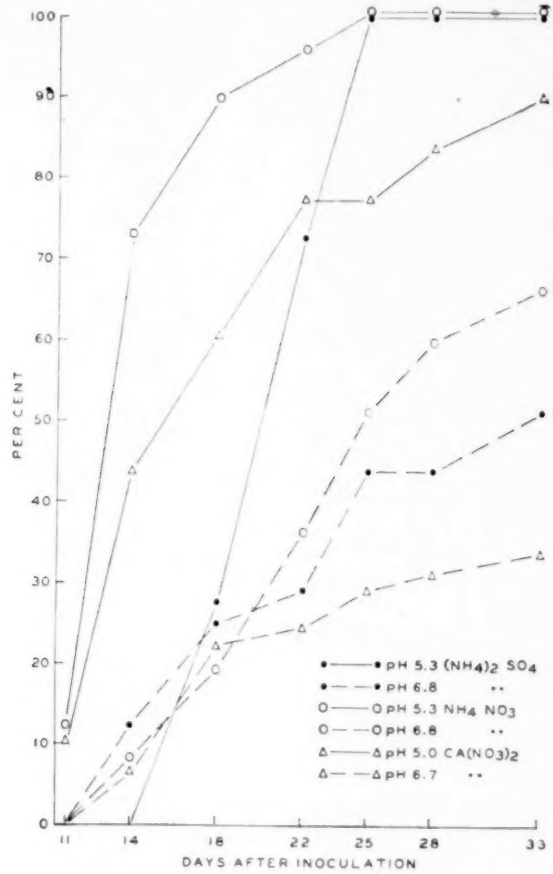

FIG. 5. The percentage of severely wilted and dead plants in cultures of Series 2 on the indicated number of days after inoculation. Inoculated Sept. 24, 1943.

in high pH calcium nitrate solutions (Comparison 5) had a higher wilt index and a greater number wilted severely or dead. These differences were of probable significance for Series 1 (except on Aug. 7), of probable and high significance for Series 2, and of high significance for Series 3. Comparisons 10 and 12 between plants grown in high pH calcium nitrate solutions and low pH ammonium sulphate and ammonium nitrate solutions, respectively, also showed significantly lower wilt indices and a smaller number of severely wilted and dead plants for the high pH calcium nitrate solutions,

except on Aug. 7 in Series 1 and on Oct. 12 in Series 2, comparison 10. The lack of significant differences on Aug. 7 and Oct. 12 was probably due to the slower development of wilt symptoms in ammonium sulphate solutions previously mentioned. There was, then, as shown in comparisons 5, 10, and 12, a consistently lower wilt index and lower wilt mortality in high pH calcium nitrate solutions than in low pH solutions in which the nitrogen was derived from calcium nitrate, ammonium nitrate, and ammonium sulphate.

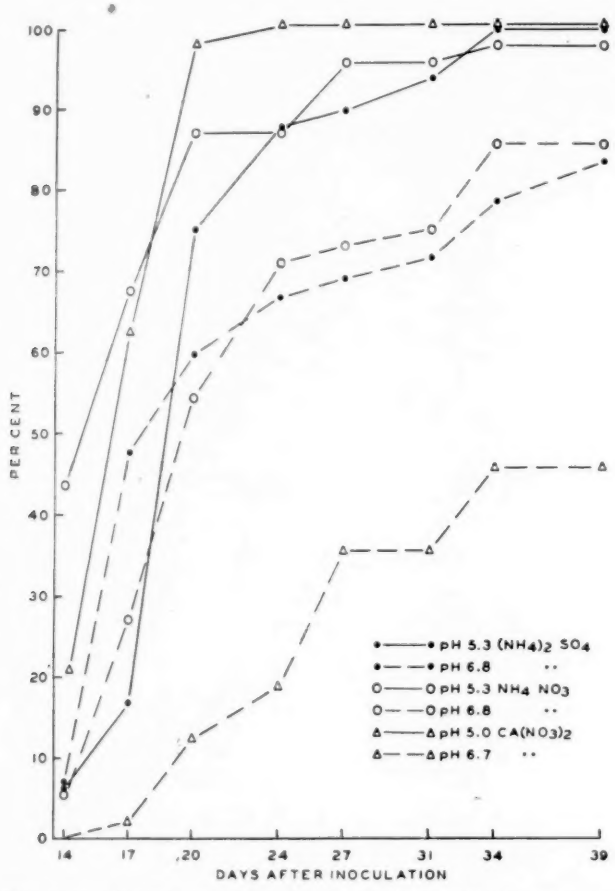

FIG. 6. The percentage of severely wilted and dead plants in cultures of Series 3 on the indicated number of days after inoculation. Inoculated Aug. 26, 1944.

If the wilt indices and the percentage of severely wilted and dead plants in the high pH calcium nitrate solutions are compared with those for the high pH ammonium sulphate solutions, as in comparison 11, it will be noted that in Series 1 and 3 the plants of greater age in the former solution were significantly lower as to both wilt index and the number of severely wilted and dead plants than those in the latter solution. The lack of significant differences in Series 2 was probably due to the relatively large number of

plants in the high pH ammonium sulphate solutions that, for some unknown reason, escaped infection (table 2). Because of the slower development of wilt in plants grown in ammonium sulphate solution, the plants of intermediate age in Series 1, 2, and 3, respectively, did not show significant differences on Aug. 7, Oct. 12, and Sept. 15. In comparison 13, the high pH calcium nitrate plants of Series 3 were significantly lower in wilt index and severely wilted and dead plants than the high pH ammonium nitrate plants, whereas in Series 1 and 2 the differences between these two treatments were not significant. In Series 3 pH changes in the solutions were adjusted so

TABLE 3.—*Analysis of variance of severely wilted and dead plants illustrating several sets of comparisons computed from the same data. Series 2, October 27, 1943*

Group comparison	Treatments	Degrees of freedom	Mean square
	TOTAL	17	1070.257
A	Low pH – High pH	1	9507.745**
	$Ca(NO_3)_2 - (NH_4)_2SO_4$	1	631.040
	$2[NH_4NO_3] - [Ca(NO_3)_2 + (NH_4)_2SO_4]$	1	813.961
	(pH × Solution) $Ca(NO_3)_2 - (NH_4)_2SO_4$	1	50.102
	(Interaction) $2(NH_4NO_3) - [Ca(NO_3)_2 + (NH_4)_2SO_4]$	1	328.093
	ERROR	12	571.952
B	Low pH – High pH	1	9507.745**
	Low pH $Ca(NO_3)_2$ – Low pH $(NH_4)_2SO_4$	1	162.760
	High pH $Ca(NO_3)_2$ – High pH $(NH_4)_2SO_4$	1	518.382
	2(Low pH NH_4NO_3) – [Low pH $Ca(NO_3)_2$ + Low pH $(NH_4)_2SO_4$]	1	54.253
	2(High pH NH_4NO_3) – [High pH $Ca(NO_3)_2$ + High pH $(NH_4)_2SO_4$]	1	1087.800
	ERROR	12	571.952
C	Low pH $Ca(NO_3)_2$ – High pH $Ca(NO_3)_2$	1	4719.132*
	Low pH $(NH_4)_2SO_4$ – High pH $(NH_4)_2SO_4$	1	3444.010*
	Low pH NH_4NO_3 – High pH NH_4NO_3	1	1722.798
	$Ca(NO_3)_2 - (NH_4)_2SO_4$	1	631.040
	$2(NH_4NO_3) - [Ca(NO_3)_2 + (NH_4)_2SO_4]$	1	813.961
	ERROR	12	571.952

* Indicates significance at the 5 per cent level.
** Indicates significance at the 1 per cent level.

that the pH level was higher than in Series 1 and 2 where the pH decreased between solution renewals. In the higher pH of the solutions of Series 3 wilt susceptibility and wilt mortality increased in the ammonium nitrate solutions.

If the differences discussed in comparisons 1, 11, and 13 were due to the source of nitrogen at the respective pH level of the nutrient solutions used and were not due to the particular balance of the nutrient salts, it follows that two factors, nitrate nitrogen and high pH, both contributed to decreased wilt incidence and plant mortality and that neither factor, by itself, would account for the observed differences. In this connection it has been reported by Taubenhaus and coworkers (8) that wilt was less prevalent in Texas soils of high pH. In regard to the possibility that the response of the plants to inoculation with the wilt organism was a nutrient

TABLE 4.—*Comparative effects of pH and nutrients upon the severity of wilt at an intermediate and at the final date of observation of each series*

Comparison	Comparison No.	Series 1			Series 2				Series 3 (1944)			
		Aug. 7	Aug. 26		Oct. 12		Oct. 27		Sept. 15		Oct. 4	
		Wilt index	Severe wilt and dead	Wilt index	Severe wilt and dead	Wilt index	Severe wilt and dead	Wilt index	Severe wilt and dead	Wilt index	Severe wilt and dead	Wilt index
Low pH – High pH	1	+[a]	+	+	++	++	++	++	++	++	++	++
$Ca(NO_3)_2$ – $(NH_4)_2SO_4$	2		–	–							–	
$Ca(NO_3)_2$ – NH_4NO_3	3											–
$(NH_4)_2SO_4$ – NH_4NO_3	4				– –	–						
Low pH $Ca(NO_3)_2$ – High pH $Ca(NO_3)_2$	5	+	+	+	+	++	++	++	++	++	++	++
Low pH $Ca(NO_3)_2$ – Low pH $(NH_4)_2SO_4$	6				+	+						
Low pH $Ca(NO_3)_2$ – Low pH NH_4NO_3	7											
Low pH $Ca(NO_3)_2$ – High pH $(NH_4)_2SO_4$	8				+	++		+				
Low pH $Ca(NO_3)_2$ – High pH NH_4NO_3	9				+	++						
High pH $Ca(NO_3)_2$ – Low pH $(NH_4)_2SO_4$	10		– –	–			– –	– –	–	– –	– –	– –
High pH $Ca(NO_3)_2$ – High pH $(NH_4)_2SO_4$	11		– –	– –						–	– –	–
High pH $Ca(NO_3)_2$ – Low pH NH_4NO_3	12	–	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –
High pH $Ca(NO_3)_2$ – High pH NH_4NO_3	13									–	– –	– –
Low pH $(NH_4)_2SO_4$ – High pH $(NH_4)_2SO_4$	14						+	+				
Low pH $(NH_4)_2SO_4$ – Low pH NH_4NO_3	15				– –	– –						
Low pH $(NH_4)_2SO_4$ – High pH NH_4NO_3	16											
High pH $(NH_4)_2SO_4$ – Low pH NH_4NO_3	17				– –	– –	–	–				
High pH $(NH_4)_2SO_4$ – High pH NH_4NO_3	18											
Low pH NH_4NO_3 – High pH NH_4NO_3	19				++	++						

[a] Plus and minus signs are used to designate the solution in each comparison in which wilt severity was significantly greater than that observed in the other solution. Plus signs indicate greater wilt severity for the first member of the comparison; minus signs, the second member. A single plus or minus sign indicates significance at the 5 per cent level; double signs, at the 1 per cent level.

balance effect, definite information might be obtained by noting the effects of nitrate nitrogen at a pH of 6.6 to 7.0 over wide ranges of nutrient salt balance. It has been shown, however, that with the balance of nutrients in the solutions used in this investigation a supply of nitrate ions derived from calcium nitrate at a pH of 6.6 to 7.0 together with an absence of ammonium ions markedly influenced wilt susceptibility and plant mortality.

There are several other comparisons in which significant differences were limited to Series 2. Comparisons 14 and 17 show a lower wilt index and less severe wilt for plants in high pH ammonium sulphate solutions, doubtless due to the number of plants in this series that escaped infection. The differences shown in comparisons 6, 8, and 15 are due to the relatively slower development of wilt symptoms in plants grown in ammonium sulphate solutions (observed in all series) which attained statistical significance only in Series 2. In comparisons 9 and 19 only the differences observed on Oct. 12 in Series 2 were due to a delay in appearance of wilt symptoms after inoculation of plants in high pH ammonium nitrate solutions. This delay was also observed in Series 3, but was not statistically significant.

The data indicate that at a pH near neutrality, wilt resistance increased only when nitrogen was derived from calcium nitrate in solutions of the nutrient balance used in these experiments.

SUMMARY

1. Plants grown in high pH calcium nitrate solutions had markedly less wilt injury and a lower mortality than those grown in any other solution.

2. On plants grown in low pH calcium nitrate solutions severe wilt symptoms developed in a shorter time than in any other solution.

3. Severe wilt injury developed rapidly on plants in low pH ammonium nitrate solutions.

4. Wilt injury in the high pH ammonium nitrate solutions was less than in the low pH ammonium nitrate solutions but was greater than in the high pH calcium nitrate solutions.

5. Wilt injury developed more slowly on plants grown in solutions containing only nitrogen from ammonium sulphate than on those grown in low pH calcium nitrate or ammonium nitrate solutions.

6. Two mutually dependent factors, a pH of 6.6–7.0 and nitrate nitrogen derived from calcium nitrate at the particular nutrient balance used, contributed significantly to increased wilt resistance and decreased wilt injury.

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ATTEMPTS TO CONTROL BACTERIAL BLIGHTS OF PEAR AND WALNUT WITH PENICILLIN

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(Accepted for publication May 28, 1946)

It is well known that penicillin, primarily bacteriostatic against Gram-positive bacteria, may also inhibit the growth of certain Gram-negative organisms. Brown and Boyle[1] reported that the penicillin sensitivity of the Gram-negative organism of crown gall, *Agrobacterium tumefaciens* (Smith and Townsend) Conn[2] syn. *Phytomonas tumefaciens* Bergey *et al.* differed little, if at all, from that of *Micrococcus pyogenes* var. *aureus* (Rosenbach) Zoph. syn. *Staphylococcus aureus* Rosenbach, the organism generally used to determine the assay value of penicillin. But they themselves point out that the conditions under which their experiments were conducted may leave the results open to question. They also found weak solutions of penicillin, even in an unpurified state, to be distinctly effective *in vivo* against crown gall on *Bryophyllum* when the galls were wrapped with cotton soaked in the penicillin solution and punctured with needles pushed through the wet cotton.[1, 3] In earlier studies by Brown and Boyle penicillin was effective *in vitro* against *Erwinia carnegieana* Standring, the Gram-positive pathogen responsible for the rot of the giant cactus (*Carnegieana gigantea* Britt. and Rose) in the Pacific Southwest. They also obtained some evidence of susceptibility of *Corynebacterium sepedonicum* (Spieckermann and Kothoff) Skaptason and Burkholder, the potato-ring-rot pathogen, to penicillin *in vitro*.[4] Waksman[5, 6] and his associates also have contributed additional information on the effect of several antibiotics on various plant pathogens.

EXPERIMENTS AT THE DECIDUOUS FRUIT FIELD STATION

In the fall of 1944, experiments were undertaken to test the antibiotic activity of unpurified penicillin against two important Gram-negative plant pathogens, namely *Xanthomonas juglandis* (Pierce) Dowson, syn. *Phytomonas juglandis* Bergey *et al.* [walnut blight] and *Erwinia amylovora*

[1] Brown, J. G., and Alice M. Boyle. Application of penicillin to crown gall. Phytopath. **35**: 521–524. 1945.

[2] The nomenclature for all bacteria referred to in this paper is that used in the sixth edition of Bergey's Manual of Determinative Bacteriology now in press, and was kindly furnished by Dr. Robert S. Breed, Chairman, Board of Editors, New York State Experiment Station, Geneva, N. Y. If the nomenclature is being used in the sixth edition of the Manual for the first time, the latest accepted synonym used in the fifth edition is also given in this paper.

[3] Brown, J. G., and Alice M. Boyle. Penicillin treatment of crown gall. Science **100**: 528. 1944.

[4] Brown, J. G., and Alice M. Boyle. Effect of penicillin on a plant pathogen. Phytopath. **34**: 760–761. 1944.

[5] Waksman, Selman A. Microbial antagonisms and antibiotic substances. 350 pp. New York Commonwealth Fund. 1945.

[6] Waksman, Selman A., Elizabeth Bugie, and H. Christine Reilly. Bacteriostatic and bactericidal properties of antibiotic substances, with special reference to plant-pathogenic bacteria. Bul. Torrey Bot. Club **71**: 107–121. 1944.

(Burrill) Bergey *et al.* [pear blight]. An improved strain of *Penicillium notatum* (1249–b–21), originally obtained from the Northern Regional Research Laboratory, Peoria, Illinois, was grown upon a modified Czapek-Dox liquid medium supplemented with lactose and corn steep liquor. The flat-bottle method was employed, and all known requirements for the maximum production of penicillin with regards to the length of time for cultivating the fungus, the pH, temperature, etc., were observed. In replicated tests the assays were sufficiently high to markedly inhibit growth of *Micrococcus pyogenes* var. *aureus*. The strain used was obtained from the Food and Drug Administration of the Federal Security Agency and was one developed by them for assay purposes.

Dr. C. E. Clifton[7] and his associates devised a convenient assay method, not heretofore reported, which obviates the use of glass assay cylinders or paper discs. Briefly the method is as follows: exactly 30 ml. of a suitable 2 per cent nutrient agar are poured into standard Petri plates of 100-mm. diameter and seeded in any one of the several conventional ways with 0.1 ml. of a 1 to 10 dilution of a 24-hour culture of *Micrococcus pyogenes* var. *aureus*. Holes exactly 10 mm. in diameter are cut in the seeded medium with any sharp-edge, sterile, metal tube, such as a cork borer. Into a hole is dropped 0.1 ml. of the penicillin-containing broth to be assayed, after which the plates are incubated for 24 hours at 35–37° C., the optimum temperature for growth of the organism. The number of units of penicillin per milliliter of the test solution is determined by referring the measurement of the diameter of the circular area of inhibition in the plate to a table of known value. *Xanthomonas juglandis* tested against the unpurified penicillin in this manner seemed to be inhibited to a slight extent, but the growth of *Erwinia amylovora* was not affected. Both of these organisms were incubated at 30° C., the temperature generally considered to be their optimum. Also, cultures of *Xanthomonas juglandis*, somewhat older than 24 hours and with maximum turbidity, were used to seed the plates, since this organism grows rather slowly even at its optimum temperature.

This method also permits of determining the number of units of commercial penicillin required to bring about complete inhibition of the growth of any susceptible organism by introducing graduated dilutions of the drug, the assay value of which is known, into the plates seeded with the organism.

Antibiotic Activity of Commercial Penicillin Against Erwinia amylovora in Vitro. Experiments with unpurified penicillin were discontinued when public sale of the pure, commercially prepared drug was permitted by government directive on March 15, 1945. Soon after that date the writer entered into a cooperative arrangement with the Commercial Solvents Corporation of Terre Haute, Indiana, which supplied generous quantities of pure penicillin of known assay value for additional studies, and for which grateful acknowledgment is made here.

[7] The writer is deeply indebted to Dr. Clifton, Department of Bacteriology, Stanford University, for many helpful suggestions and criticisms during the course of the work and in the correction of the manuscript.

Strains of *Erwinia amylovora* from different sources were tested against graduated dilutions of commercial penicillin in sterile, distilled water by the plate method described. Certain autogenous cultures from pear trees and others from apple trees in the same orchard were sensitive to the penicillin; all other cultures were resistant to its action. The circular areas of inhibition in the plates were clean-cut, but all measurements were made under the microscope when it was observed that minute colonies of the organism, imperceptible to the naked eye, slowly grew in a narrow zone, usually about 5 mm. wide, between the area of absolute inhibition and that of unrestricted growth. Under the microscope, the organism growing in these marginal zones appeared either as tiny, discrete colonies or as scarcely more than a granulation in the agar. Throughout this paper the diameter of the zones of inhibition as determined by both microscopic and macroscopic inspection are given, the first figure representing the diameter as observed under the microscope and the second as measured directly with the naked eye.

Dilutions of commercial penicillin containing 1000 units per 0.1 ml. inhibited the growth of the autogenous strains of *Erwinia amylovora* in circular areas of 35 to 45 mm. diameter. Dilutions of 100 units per 0.1 ml. inhibited growth in circular areas varying from 25 to 35 mm. Dilutions of 10 units per 0.1 ml. failed to affect the organism at all.

In parallel experiments with *Erwinia amylovora* from apple tested against the same lot of penicillin, a slightly greater resistance to the drug was noted; thus dilutions of 100 units per 0.1 ml. inhibited growth in circular areas of only 20 to 30 mm. diameter.

In check plates of *Micrococcus pyogenes* var. *aureus*, dilutions of the same lot of penicillin containing only 0.1 unit per 0.1 ml. inhibited growth in circular areas with diameters varying from 25 to 35 mm.

Antibiotic Activity of Commercial Penicillin Against Xanthomonas juglandis in Vitro. The walnut-blight organism proved to be definitely more susceptible to penicillin than *Erwinia amylovora*. Whereas a dilution of the drug containing 10 units per 0.1 ml. failed to affect the *Erwinia*, the growth of *Xanthomonas juglandis* in replicated tests was inhibited in circular areas of 20 to 30 mm. diameter. At dilutions of 1 unit per 0.1 ml. the organism was not affected.

That penicillin is not only bacteriostatic in its action against *Xanthomonas juglandis* and *Erwinia amylovora*, but bactericidal as well, is evidenced by the fact that no colonies of the organism ever developed in the circular areas of complete inhibition regardless of time. Numerous subcultures made at frequent intervals for two weeks from such areas in plates of *Xanthomonas juglandis* remained sterile.

Attempts to Control Erwinia amylovora in Vivo. Four mature Bartlett pear trees severely affected with blight were injected with penicillin solution in June, 1945. Infection had taken place several months earlier, almost exclusively through the blossoms as is characteristic of the disease in the Santa Clara Valley, and had spread to the twigs and limbs where it was

still very active. Four holes, $\frac{7}{16}$ inches in diameter and not less than $3\frac{1}{2}$ inches deep, were bored just below the crotch of a tree and equidistant on the circumference of the trunk which averaged 22 inches. Pieces of galvanized iron tubing, $\frac{7}{16}$ inches in diameter and $2\frac{1}{2}$ inches long, were driven just deep enough into the holes to insure a tight seal. Five-foot lengths of rubber hose attached to the projecting ends of the tubes conveyed the penicillin solution in a straight drop from clean cans suspended in the trees. The cans were ventilated through cotton filters in the lids.

One tree was injected with 500 ml. of a solution of commercial penicillin containing slightly in excess of 1000 units per ml. It was this dilution, 0.1 ml. of which inhibited growth of *Erwinia amylovora* in circular areas 25 to 35 mm. in diameter in test plates. The drug was dissolved in distilled water near the freezing point to offset possible deterioration by the warm atmospheric temperatures prevailing at the time. The three other trees received identical dilutions of the drug prepared in the same way, but only 250 ml. of solution per tree were used.

The trees showed no unfavorable reaction to the drug. The solution was readily absorbed by all four trees within a few hours, but the results were unqualified failures. A day after injecting the trees, bits of diseased wood from twigs selected as close to the point of injection as possible, were planted in sterile, green, pear-fruit tissue and incubated at 30° C. *Erwinia amylovora* grew luxuriantly in practically 100 per cent of the cultures prepared in this way.

A fifth tree received protracted treatment with a stronger solution containing 1408 units of commercial penicillin per ml. The drug was pure, but, unlike the commercial product, it had not gone through the final process of sterilization. In this condition it is much cheaper but is considered unsuited for therapeutic use in humans or animals. The tree was injected three times at intervals of 24 hours, each time with 500 ml. of the solution. Following the injections, several hours after the solution had been absorbed, one liter of sterile, distilled water was added to the can for the sole purpose of keeping the wood in an absorptive condition, experience obtained in preliminary experiments having shown that if considerable time is allowed to elapse between injections the wood ceases to absorb the solution. This may possibly be due to the formation of tyloses or the production of gum in the tracheae.

The result of this experiment was an unqualified failure. Actually more of the numerous cultures made from the treated wood yielded the organism than did wood from check trees adjoining. This must be regarded as a coincidence.

Attempts to Control Xanthomonas juglandis in Vivo. In April, 1945, a total of 11 mature Payne (English) walnut trees with an average diameter of 29 inches growing near the Deciduous Fruit Field Station were injected with commercial penicillin in solution prepared in precisely the same way as described above. No less than 4 and usually 8 injections were made per tree depending upon the circumference of the trunks.

Plate tests reported elsewhere in this paper showed that 0.1 ml. of a dilution of the drug containing 100 units per ml. was sufficient to inhibit growth of *Xanthomonas juglandis* in large areas. The strength of the solution used in the trees varied from 200 to 600 units per ml., and each tree received 1 liter of solution.

The results were unqualified failures. Broth cultures made from occasional lesions on the petioles of leaves taken as close to the points of injection as possible yielded the organism readily.

The Effect of Passage Through Pear Wood on Penicillin. To determine whether the failure of penicillin to control *Erwinia amylovora in vivo* might have been due to adsorption or inactivation of the drug by the wood in which it was injected, the following experiment was performed.

A healthy, 3-year-old pear shoot, 1 inch in diameter and 32 inches long, was cut from a dormant tree in January and painted with melted paraffin except at the cut ends. The shoot was then forced through 2 large, perforated rubber corks, one of which served to seal about 10 inches of the shoot inside a Martin vacuum jar. The cork at the opposite end of the shoot fitted into a receptacle holding 20 ml. penicillin solution in which 200,000 units of the drug were dissolved. The Martin jar was evacuated under low vacuum, and the liquid that was extracted from the end of the shoot was removed from time to time. When the last of the penicillin solution had disappeared into the shoot, the pump was kept running for several minutes, and then an indeterminate amount of distilled water was added to the receptacle and drawn through the wood by vacuum.

At the beginning of the experiment, pure, colorless sap started to drip from the end of the shoot in the Martin jar almost as soon as the vacuum was applied. After 25 ml. were collected, a faint suggestion of yellow color in the liquid indicated that some penicillin was entering the sap stream. This extract was discarded and the vacuum pump started again. By the time an additional 50 ml. of liquid were collected, the color of the solution dripping from the shoot was practically the same as that of the original solution, a sample of which had been kept. This extract was also discarded. The pump was started again and 20 ml. of liquid extracted, the deep yellow color of which was only slightly lighter than that of the original penicillin solution. This liquid was used in the following experiments.

A series of 1 to 10 dilutions were made from each of the following: (1) the original penicillin solution; (2) the dark yellow extract from the wood; (3) the same extract from wood after it had been filtered under vacuum through a short No. 3 Chamberland candle. The antibiotic activity of the three series of dilutions was tested at the optimum temperature of the organisms in plates seeded with *Micrococcus pyogenes* var. *aureus* and *Xanthomonas juglandis.*

The number of units of penicillin present was known for only the first of the three series of dilutions, namely that made from the original, unfiltered penicillin solution. This series of 1 to 10 dilutions contained 10,000;

1000; 100; 10 units per ml. A parallel series of dilutions made of the yellow extract from wood had somewhat less antibiotic activity against *Micrococcus pyogenes* var. *aureus* in test plates than the original unfiltered penicillin series. But the difference seemed entirely a matter of dilution rather than inactivation of the drug. Thus, 0.1 ml. of the yellow extract just as it came from the wood inhibited growth of the bacteria in circular areas of 60–70 mm. diameter. As the amount of penicillin decreased with each succeeding dilution, the areas of inhibition, always sharply defined, grew correspondingly smaller. Nevertheless, even the fourth dilution in the series inhibited growth in clean-cut areas of 20–30 mm. diameter.

Passage of the yellow extract from the wood through the Chamberland candle reduced its antibiotic properties still further. Before passage through the candle, the undiluted extract inhibited growth of *Micrococcus pyogenes* var. *aureus* in circular areas of 60–70 mm. diameter. After filtration the diameters of the areas of inhibition were only 46–60 mm.

Growth of *Xanthomonas juglandis* was inhibited in clean-cut, circular areas of 30–40 mm. diameter by the extract as it came from the wood. But a 1 to 10 dilution of the extract failed to affect the bacteria at all except at the edges of the holes in the plates. At these points the growth of the colonies was feeble, and they remained very small.

DISCUSSION

There can be no doubt whatever that growth of certain strains of the Gram-negative organisms *Erwinia amylovora* and *Xanthomonas juglandis* can be inhibited *in vitro* by penicillin if strong enough solutions be used. That the action of the drug is not only bacteriostatic but bactericidal as well was established. The fact that in preliminary phases of the work certain strains of *Erwinia amylovora* proved resistant to strong concentration of penicillin might be attributed to the use of penicillin material that had deteriorated somewhat; before arrangements were completed with the Commerical Solvents Corporation for supplies of the drug, a certain amount of it was purchased in local drug stores. The conditions under which it was kept before purchase were not known, but it is recognized that penicillin slowly deteriorates with age even under the best conditions of storage and rapidly under improper conditions, especially, with improper temperature.

There are several possible reasons why penicillin failed utterly to control *Erwinia amylovora* and *Xanthomonas juglandis in vivo:* (1) too small amounts of penicillin solution may have been used, even though its strength was always considerably in excess of the concentrations required to destroy either organism *in vitro;* (2) the drug may have been diluted so much by the sap of the trees in which it was injected that it was rendered harmless; (3) the penicillin may have failed to reach the bacteria in the disorganized tissues of the lesions; (4) more injections at shorter intervals may have been necessary; (5) the action of penicillin may be evident only when in contact with susceptible, actively growing bacteria over a period of time.

With reference to the first reason cited, namely, that too small amounts of the drug may have been injected, it should be stated that at no time was the disinfection of an entire tree expected or contemplated. The high price of penicillin makes experiments of such scope impractical. Even its cost in the small scale tests was prohibitive from the growers' standpoint. For instance, the retail cost of penicillin at the time was $3.50 per 100,000 units. The cost of injecting 1 walnut tree experimentally with 600,000 units was $21.00 for the drug alone. The retail price has steadily dropped since then, and at the time of writing this paper penicillin sells for $1.25 per 100,000 units. Even this price makes its use impractical by growers except in amounts even smaller than those used unsuccessfully in the experiments reported.

It was hoped that the drug might reach and permeate the diseased tissue of some lesions comparatively close to the points of injection in sufficient concentration to kill the bacteria in them. Had this been accomplished, the method of control on a greater scale could be referred to that probable, future day when the cost of the drug, even in large amounts, would be no deterrent to its use.

That the penicillin probably did not reach the bacteria in sufficient concentration to kill them is indicated by the fact that a pear shoot 32 inches long and 1 inch wide yielded 25 ml. of pure sap to begin with, then 50 ml. of sap containing penicillin in ever-increasing amounts but never as much as in the original solution. Actually the original solution (20 ml.) came through the wood largely as a unit; the color of a final 20 ml. of extract indicated this, and its antibiotic activity in plate tests further substantiated it. It is clear that the total sap content of the pear shoot amounted to well over 3 times the original volume of penicillin solution used, a factor that probably entered in the injection experiments and vitiated them. Whereas, the penicillin solution could be pulled through the wood by vacuum largely as a unit in a few minutes, a matter of hours was required to inject a tree during which time the penicillin solution, gradually entering the sap stream, probably was diluted to a point of ineffectiveness.

Logically, for more precise results, the injections should have been made in one of the many scaffold branches of a tree rather than in its trunk, the considerably greater size of which made proportionally greater dilution of the drug injected into it inevitable. Better still, the drug might have been injected into a single diseased twig or shoot or even pulled through one by vacuum, after which cultures made from the wood would have revealed whether the drug had killed the organism or not. But, results obtained in these ways would have little practical application regardless of their possible success. From the beginning it was recognized that control of the two organisms *in vivo*, to have any practical significance, would have to be through the tree trunks. To attempt to inject individual scaffold branches every year would be impractical for growers. Aside from the great amount of time, labor, and equipment that would be required to treat an orchard in

this way, the problem of keeping the wounds sterile until healed to prevent wood decays, by no means simple even when the trunks alone are injected, would become too greatly complicated were it necessary to bore all the scaffold branches with holes. A considerable acreage of pears growing on calcareous soils in the Santa Clara Valley must, for best results, be injected with iron salts every two or three years to correct lime-induced chlorosis. The injections are made in the trunks. Heart rots following such treatment are by no means uncommon. The expansion and contraction of the wood under fluctuating temperatures, as well as the movement of the trees in the wind alone might be sufficient to break the seal between the wood and the wax with which the holes are usually plugged and permit organisms of decay to make their way inside. These same factors would probably make it even more difficult to keep the wounds in scaffold branches sterile until healing was complete.

Whether the diseased wood, unlike healthy wood, resisted passage of the penicillin through it or adsorbed or inactivated the drug is not known. Nor is there any assurance that more injections at shorter intervals might not have produced better results, but again the problem of time, labor, and expense from the growers' standpoint, discouraged the undertaking of such experiments. Lastly, whether the penicillin remained in contact with the organisms long enough to kill them is problematical.

SUMMARY

The antibiotic properties of penicillin were tested against the Gram-negative organisms, *Erwinia amylovora* (Burrill) Bergey *et al.* [pear blight] and *Xanthomonas juglandis* (Pierce) Dowson [walnut blight] *in vitro* and *in vivo*. In conventional plate tests, dilutions of the drug in distilled water at the rate of 100 units per 0.1 ml. inhibited growth of *Erwinia amylovora* completely in circular areas of 25 mm. diameter, as seen under the microscope, or 35 mm., as seen by the naked eye. At the rate of 10 units per 0.1 ml., the organism was not affected. *Xanthomonas juglandis* is more susceptible and was inhibited by dilutions of 10 units per 0.1 ml. in circular areas of 20 to 30 mm. diameter, but it was not affected by 1 unit per 0.1 ml.

Penicillin is not only bacteriostatic but bactericidal as well.

Attempts to control either organism *in vivo* failed completely. Individual pear trees, severely affected with blight, were injected once in their trunks with as much as 500 ml. of penicillin solution containing more than 1000 units per ml. Also, one tree was injected three times, at 24-hour intervals, with 500 ml. penicillin solution containing 1408 units per ml. Numerous cultures made at intervals from active lesions on twigs close to the points of injection showed that no control of the organism had been obtained.

Individual walnut trees were injected with 1 liter of penicillin solution containing 200 to 600 units per ml. with no better success.

To determine whether the penicillin solution might have been adsorbed

or inactivated by the wood, 20 ml. of solution containing 200,000 units were drawn through a 32-inch long, healthy, 3-year-old pear shoot under vacuum. The wood yielded practically 75 ml. of sap before the penicillin solution came through. Finally 20 ml. of extract were collected, the color of which was only slightly lighter than that of the original solution. Its antibiotic properties against *Micrococcus pyogenes* var. *aureus* and *Xanthomonas juglandis* were found to be slightly impaired, but by dilution rather than inactivation. Passage of the extract through a short No. 3 Chamberland candle reduced its antibiotic activity slightly.

The failure of penicillin to control *Erwinia amylovora* and *Xanthomonas juglandis in vivo*, even at short distances from points of injection in the wood, is believed to have been due largely to excessive dilution of the drug by the sap. Other possible reasons for the failure are discussed.

The present prohibitive cost of penicillin makes it impractical to use larger amounts or stronger solutions of it than reported, and even if either organism had been controlled *in vivo*, growers still would have to wait until the drug is considerably cheaper before such a method of control could be utilized economically.

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A STUDY OF VEGETABLE SEED PROTECTANTS[1]

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Chemical treatment of vegetable seeds to protect against the ravages of damping-off has been the subject of much investigation during the past two decades. While a considerable amount of research had been done earlier, the introduction of the practice of dust treatment of vegetable seeds in the late twenties stimulated greater activity.

Under the stress of war-imposed scarcities of certain metallic salts heretofore principally used, a study of less-scarce metals and of certain newly developed organic fungicides was undertaken in the spring of 1942. Results obtained in the preliminary trials conducted by the junior author prior to his entrance into the armed services led to the detailed study of a few materials by the senior author during the winter of 1942–43. The studies herein reported are additional to tests conducted in cooperation with the committee of the American Phytopathological Society on coordination in cereal and vegetable seed treatment research.

THE DEVELOPMENT OF DUST MATERIALS AS SEED PROTECTANTS

Organic mercury compounds, already in use for a decade in liquid form, were developed for use as dust treatments on vegetable seeds in the mid-twenties. In the United States, by 1926–27, Orton (31), Coons and Stewart (8), Jones (25), and Haenseler (14) were able to report success with these dust materials as seed protectants on vegetables. Although McWhorter (30) found the dust treatment less effective than the comparable liquid treatments in protecting beets against soil-borne pathogens in greenhouse trials, the development of suitable seed protectants in dust form constituted an immense step toward wide adoption of the practice of protective seed treatment. In most cases satisfactorily replacing the more inconvenient method of soaking the seed in liquid solutions, they proved a boon in the struggle against damping-off.

The soaking of vegetable seeds in solutions of copper sulphate had long been practiced with good results by some growers. The ease of application of dusts to the dry seed led Coons and Stewart (8) and Horsfall (17, 18) to test dusting with powdered copper sulphate and monohydrated copper sulphate as a substitute for the method of soaking the seeds in copper sulphate solution. These copper salts were found to adhere poorly to the seed coat and proved to be rather unsatisfactory in performance. However, recognition of the poor adherence as a probable cause of the poor protection stimulated a survey of the other salts of copper, from among which Horsfall (18,

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[2] Associate plant pathologist and research assistant, respectively, West Virginia Agricultural Experiment Station.

19) selected red cuprous oxide as being the most promising. Subsequent work by Horsfall (20, 21, 22, 23) and by others (3, 5, 6, 7, 26, 33) has shown the benefits to be derived from the use of this material and also has revealed certain difficulties arising from its use. It has proved to be injurious on many acid soils and, in common with other copper salts, it frequently causes a hardening of the seed coats of lima beans. It is also injurious to crucifers. To reduce interfacial friction between seeds, it has been found necessary to add graphite with the red cuprous oxide (4, 5, 22), a practice which Hulbert and Whitney (24) had found efficacious in reducing interfacial friction between pea seeds treated with Ceresan or with copper carbonate.

A later development was use of the yellow form of cuprous oxide, the color change being the result of reduction in particle size. The yellow cuprous oxide has been more effective than the red form at equal dosages and has been equally effective at reduced dosages (16, 22).

Zinc oxide was found effective as a seed treatment material by Horsfall (20, 21) and was popularized for use on spinach by Cook and Callenbach (5, 6, 7). Other than on spinach and cabbage it has been used to a much less extent than has cuprous oxide. Treatment with zinc oxide also increases the friction between seeds, thus necessitating the addition of graphite where the seeds are to be machine-planted.

The adoption of the oxides of these two metals as seed-protectant materials did much to popularize the practice of treatment of vegetable seed. These materials, relatively inexpensive and effective on many crops and under many conditions, were adopted quickly by commercial vegetable producers. In 1935 the red cuprous oxide treatment was applied to 20,000 bushels of peas in New York (1), and in 1936 it was used on 50,000 bushels in that State alone (2). By 1937 Cook (5) reported that approximately 150,000 pounds of spinach seed were treated annually in the Norfolk, Virginia, area. Because of the popularity of the zinc oxide seed treatment in that area, presumably most of this seed was treated with zinc oxide. During the same period, these materials were accepted and recommended as seed protectants in many states and on many crops.

These developments in the field of protective seed treatments stimulated active search for other effective materials. In 1940 Cunningham and Sharvelle (9) reported promising results obtained in the treatment of lima beans with two synthetic organic compounds. One of these, later identified as tetrachloro-para-benzoquinone and marketed as Spergon (36), has proven generally to be a very satisfactory seed protectant on vegetable seeds. It has been especially successful with legumes (9, 11, 27, 36). Increased height of peas from Spergon-treated seed was observed by Felix (11). Sharvelle and Shema (35) reported evidence of stimulation in field-grown peas while McNew (28) obtained increased size of plants from disease-free peas planted in steamed soil when Spergon was used as the seed treatment material. Davis and Haenseler (10) and Gould (12) reported that, while Spergon was satisfactory with beans, lima beans, and peas, it was less effective than certain

other materials with beets and spinach. Person and Chilton (32) reported that, with flowers, emergence was satisfactory following the use of Spergon but that the final stand was lower than where yellow cuprous oxide was used.

Recognized slightly later than Spergon, two derivatives of carbamic acid, tetramethyl thiuramdisulphide and ferric dimethyldithiocarbamate, have given favorable results in studies reported on in 1943 by McNew (29), Taylor, Leach, and Rupert (37), Davis and Haenseler (10), Gould (12), and Porter (34). Many investigators have reported on these materials since 1943. During 1943 tetramethyl thiuramdisulphide was introduced as a seed-protectant material under the name "Arasan," this containing 50 per cent of the active ingredient in a non-wettable diluent. Ferric dimethyldithiocarbamate, introduced into commercial use as "Fermate," has found its chief value as a protective spray fungicide, though it is, in many cases, an effective seed protectant.

DAMPING-OFF

Throughout these studies the term damping-off has been construed as applying to the rotting of viable seeds and to the killing of the seedlings before emergence, as well as to the more commonly recognized killing of the emerged seedlings. For convenience, the terminology of Horsfall (18) has been employed to distinguish between the types of damping-off.

Pre-emergence damping-off. With this type of damping-off the young seedling is invaded and destroyed before it emerges above the surface of the soil.

Post-emergence damping-off. This is the more commonly recognized stage of damping-off. The already emerged seedling is invaded and the stem becomes water-soaked near the ground line—the plant then topples over suddenly, usually while the cotyledons are still green. With slightly older seedlings the plant occasionally survives invasion but the stem becomes discolored and hard, the so-called wire-stem of seedlings. In other cases small brown lesions may develop on the stem or roots but the seedlings survive the invasion.

The fungus *Pythium ultimum* Trow principally was responsible for the damping-off reported in the following experiments.

MATERIALS

The preliminary trials were with proprietary materials developed as seed protectants, various inorganic salts, and a few organic compounds available from fruit spray experiments. Later, other materials were obtained specifically for trial as seed protectants. All proprietary compounds were provided gratuitously by the several manufacturing companies. A list of the materials tested, together with their composition and source follows:

AAZ: Special zinc oxide; Rohm & Haas Company.

Arasan: Tetramethyl thiuramdisulphide, 50 per cent active material, non-wettable; E. I. duPont de Nemours & Company.

C–119: Copper trichlorphenate, 20 per cent; Dow Chemical Company.

Cuprocide: Red cuprous oxide; Rohm & Haas Company.

Fermate: Ferric dimethyldithiocarbamate, 70 per cent active material, wettable; E. I. duPont de Nemours & Company.

Iron sulphate; Mellon Institute.

Japanese Beetle Spray: Tetramethyl thiuramdisulphide, 80 per cent active material, wettable; E. I. duPont de Nemours & Company.

Phenothiazine, micronized; E. I. duPont de Nemours & Company.

Semesan: Hydroxymercurichlorophenol, 30 per cent; E. I. duPont de Nemours & Company.

Spergon: Tetrachloro-para-benzoquinone; Naugatuck Chem. Div., U. S. Rubber Co.

Tetrachlororesorcinol; Pennsylvania Salt Company.

Thiosan (now Tersan): Tetramethyl thiuramdisulphide, 50 per cent active material, wettable; E. I. duPont de Nemours & Company.

In addition to the above, boric acid, copper carbonate, silver carbonate, silver chloride, silver chromate, silver iodide, silver nitrate, silver orthophosphate, silver oxide, and silver sulphate, C.P. grade, were tested.

PRELIMINARY TRIALS

In a preliminary survey in 1942 the protective value of a number of materials was tested on spinach seeds in one or more of four greenhouse

TABLE 1.—*Preliminary trials on seed treatment of spinach, 100 seeds per plot, with dosage at full maximum capacity of seeds*

	Emergence of plants, in per cent			
	Test 1	Test 2	Test 3	Test 4
Date planted	Apr. 26	May 15	June 4	June 29
Record, days after planting	8	7	6	7
Number of replications	5	4	5	5
Material:				
Silver carbonate	30.0[a]	60.0	11.0	41.6
Silver chloride	.....	31.8[a]	.....	.....
Do	.....	39.0[b]	.....	.....
Silver iodide	.....	28.5	.....	.....
Silver nitrate	19.2[a]	.....	.....	.....
Silver sulphate	12.2[a]	.....	.....	.....
Zinc oxide	51.4	.....	.....	.....
Copper carbonate	.....	52.2	.....	.....
Cuprocide	58.2	60.3	44.4	45.6
Iron sulphate[c]	10.4	.....	.....	67.8
Sulphur (micro)	.....	22.3	.....	.....
Semesan	.....	.....	.....	39.2
Boric acid	11.4	.....	.....	.....
Fermate	.....	72.3	65.4	59.0
Phenothiazine, micronized	.....	.....	.....	43.8
Spergon	55.2	.....	52.6	.....
T.M.D.S.	.....	.....	.....	60.6
No treatment	13.4	30.3	27.4	49.0
Least Mean Sign. Diff. 5 per cent	10.79	9.76	7.27	8.90

[a] Diluted, one part silver salt to nine parts bentonite.
[b] Diluted, one part silver salt to nine parts infusorial earth.
[c] Finely powdered $FeSO_4 \cdot 1.5H_2O$.

trials. In these tests the seeds were treated at the maximum load of material the seeds would retain, the presumably more toxic silver salts being diluted before application. Final counts, presented in table 1, were made a week or less after planting and present no data on the control of post-emergence damping-off. In fact, it is probable that additional emergence would have been recorded had later readings been made on these tests. Failure to emerge under these conditions may have been due to chemical injury of the seedlings, to pre-emergence damping-off, or to a combination of both factors.

As a result of these trials, silver carbonate, Fermate, Spergon, and T.M.D.S. (tetramethyl thiuramdisulphide, 80 per cent active grade, processed as "Jap Beetle Spray") were selected for further tests on a number of different crops.

TABLE 2.—*Percentage seedling emergence in preliminary trials on seed treatment*

	Emergence							
	In greenhouse						In field	
	Cabbage	Carrot	Lettuce 1	Lettuce 2	Muskmelon	Tomato	Sweet corn	Peas
Date planted	June 19	July 11	June 19	July 9	June 5	June 30	June 16	June 15
Days after planting	5	11	8	8	10	8	9	10
Soil pH	5.25	5.4	5.25	5.4	5.4	5.4	7.4	7.4
Material:[a]								
Fermate	93.2	51.6	9.0	15.2	70.0	88.8	92.4	89.0
T.M.D.S.	93.6	53.2	32.6	26.0	.....	88.8	94.0	86.2
Spergon	89.6	56.0	35.6	33.0	72.8	89.6	89.2	86.0
Cuprocide	74.2	53.8	29.6	39.0	70.4	88.4	85.4	88.0
Silver carb.	86.0	45.2	26.2	10.6	71.1	79.8[b]	78.6	67.2[c]
Not treated	77.6	40.0	50.0	38.0	4.4	76.2	79.4	76.0
Least Mean Sign. Diff. 5 per cent	6.95	7.85	12.37	5.60	4.42	4.92	5.76	5.49

[a] All treatments at maximum dosage with the exception of sweet corn, where 1 gram of material was applied to 180 grams of seed.

[b] Pronounced stunting of seedlings.

[c] Severe post-emergence injury to seedlings.

The results of these tests on other vegetable seeds are shown in table 2. Here Fermate, T.M.D.S., Spergon, and Cuprocide were approximately equal in effect on seedling emergence on all crops excepting lettuce, the one striking exception being the reduced stand where Cuprocide was applied to cabbage seed (an effect which has been reported many times). With lettuce the picture was less clear and no benefit was obtained from any treatment. In considering these data it should be emphasized that, except on sweet corn, all materials were applied at the maximum amount which would remain on the seed. With some kinds of seeds an excessively high dosage must have been applied, since later tests showed that lettuce would retain Fermate to the extent of approximately 12 per cent of the seed weight. It is probable

that such excessive rates of treatment were conducive to seedling injury on lettuce.

METHODS

Preliminary trials indicated differences between test materials in their ability to reduce or delay post-emergence damping-off. The following methods were employed to demonstrate such differences, and to achieve some standardization in technique.

Seed Treatment. The seeds to be treated were weighed on an analytical balance (0.1 mg. sensitivity). The test material was added to the seeds which then were shaken vigorously in a closed vial, then shaken lightly over a 40-mesh screen to remove unattached particles of the test material, and reweighed. If the desired dosage had not been reached, more material was added, shaken, and screened as before. If the desired dosage was not approximated in three attempts, a fresh start was made with untreated seeds.

This method proved satisfactory with cabbage, carrot, cucumber, lima bean, pea, and spinach seed. With lettuce it was necessary to expose the seeds for some hours in the balance room to prevent humidity differences from causing variations between successive weighings. With seeds not adjusted to the humidity of the balance room such variations frequently were greater than the desired weight of test material.

The treatment method described was unsatisfactory with tomatoes because the abrasive action of the shaking removed hairs from the seed coat and caused losses in weight; such losses varied irregularly with the amount of shaking. Here, the treatment was accomplished by dilution of the test material with talc. Dosage rates were obtained by differential dilution, applying the full load of material that the seed would retain.

Seed Selection. The spinach seed was screened and only those seeds which passed through an 8-mesh, but not through a 10-mesh, screen were used. The larger seeds thus removed gave a lower rate of germination than did the medium-size seeds. The cabbage seed was screened to remove the smallest seeds. The other kinds of seeds were not sized.

Seed-counting Procedure. The seeds were stored in vials until planted, at which time they were counted and distributed immediately into the row. Counting was facilitated by the use of a 1.5×5-inch card folded into a trough. A small quantity of seeds was poured on the card, the required number counted, and then poured from the card directly into the row. This procedure avoided a considerable amount of handling of seeds and minimized the loss of material from the seeds before planting. Distribution of the seeds in the row was corrected by moving seeds with forceps, though little of this was needed if sufficient care was exercised.

Soil. Clay loam at pH 5.4, with sufficient sand added to prevent severe crusting, was used throughout these experiments. Initially it was infested heavily with a freshly isolated culture of *Pythium ultimum*. Before starting each test the required amount of soil was screened and mixed by repeated shoveling to insure uniformity.

Where the possibility of chemical injury to the seed was anticipated, a duplicate planting was made using soil from the same source, but partially sterilized with formaldehyde according to the method of Guterman and Massey (13). This was done in order to differentiate between reduced emergence due to chemical injury and low emergence due to poor protection against pre-emergence damping-off. The method of soil sterilization proved satisfactory in controlling pre-emergence damping-off. A small amount of post-emergence damping-off occurred, but the distribution of this suggested post-planting contamination. These damped-off seedlings did not complicate the emergence data since they were detected and recorded in the daily counts.

Planting. Greater uniformity of covering of the seeds was achieved by pressing the rows to a constant depth ($\frac{5}{8}$ inch for small seeds, $\frac{1}{4}$ inch for cucumber and other large seeds). The row-press consisted of a $\frac{1}{2}$ inch board having rounded edges with sidepieces adjustable to the desired depth. After the seeds had been spaced, the row was filled with finely screened soil of the same source, dispensed from a large funnel.

Watering. The flats were watered heavily by sprinkling twice daily at a rate to maintain a high soil moisture.

Plant Counts. When an appreciable emergence had occurred, daily counts were made of all plants. Those that had damped-off were recorded and removed. This method of counting provided data on the time of development of post-emergence damping-off with different materials. In the earlier tests 100 seeds were used, each replicated to five plots in a randomized block arrangement. Later the seed number per plot was reduced to 50 with some crops, and to 20 with such crops as cucumbers. The total number of seeds used is shown in the discussion of each test.

Statistical Treatment. The least significant difference between treatment means was determined by the method of Analysis of Variance.

SEED TREATMENT OF SPINACH—MATERIALS AND DOSAGE

Following the preliminary trials, it was deemed advisable to test the materials at different dosage rates. The plants were counted daily for the first few days after emergence, later at 2-day intervals when most of the plants had attained such size that they were not likely to damp-off and "disappear" during a 48-hour period.

In two trials of spinach (planted September 3 and October 19, 1942) certain materials and dosages were duplicated; these may be considered in a combined analysis of data drawn from the two trials.

The final results of the September 3 test are shown in table 3. Here the daily counts permitted accumulation of the total number of plants damped-off. This total, when added to the number standing at any date, permits the calculation of the total emergence to that date. The difference between the total emergence of plants and the maximum expected emergence of plants (75 to 85 per cent in later trials with partially sterilized soil) may be attributed to pre-emergence damping-off.

TABLE 3.—*Results of greenhouse test on spinach planted Sept. 3, 1942. Five replications of 100 seeds each*

Material	Dosage (Per cent)[a]	Percentage of plants 16 days after planting		
		Surviving	Damped-off	Emerged
T.M.D.S.	2.76	58.6	7.8	66.4
Do	0.97	62.6	10.6	73.2
Do	0.50	55.0	10.6	65.6
Do	0.32	53.8	13.4	67.2
Do	0.12	47.8	16.0	63.8
Fermate	7.38	48.4	18.2	66.6
Do	0.54	47.2	22.0	69.2
Do	0.31	38.4	25.6	64.0
Do	0.13	41.6	18.2	59.8
Spergon	0.33	13.8	16.4	30.2
Cuprocide	2.00	20.2	36.8	57.0
No treatment	.....	8.2	11.6	19.8
Least Mean Sign. Diff.				
5 per cent	.....	9.25	8.27	7.62
1 per cent	.....	12.38	11.06	10.20

[a] Throughout this study, dosage refers to the weight of the treatment material expressed as percentage of seed weight.

In this test T.M.D.S. and Fermate, even at dosages as low as 0.1 per cent, resulted in plant stands at 16 days which were significantly higher than those where Cuprocide and Spergon were used at their recommended rates. Spergon, as used, did not significantly improve the stand over the controls. The effect of treatment on emergence of plants was essentially similar to the effect on survival of plants, an exception being found with Cuprocide which

TABLE 4.—*Results of greenhouse test on spinach planted Oct. 19, 1942. Five replications of 100 seeds each*

Material	Dosage (Per cent)	Percentage of plants					
		16 days after planting			24 days after planting		
		Surviving	Damped-off	Emerged	Surviving	Damped-off	Emerged
T.M.D.S.	2.55	60.0	7.4	67.4	53.4	14.2	67.6
Do	1.22	51.2	16.2	67.4	44.6	23.0	67.6
Do	0.51	44.6	22.2	66.8	38.6	29.0	67.6
Do	0.28	38.2	21.2	59.4	32.2	27.8	60.0
Do	0.09	45.2	17.4	62.6	38.4	24.8	63.2
Thiosan	2.80	61.0	4.2	65.2	54.4	11.0	65.4
Do	1.41	50.6	12.2	62.8	43.2	19.6	62.8
Do	0.52	49.0	20.0	69.0	42.2	26.8	69.0
Fermate	7.68	52.2	17.8	70.0	42.6	28.2	70.8
Do	0.54	34.2	26.4	60.6	28.2	32.8	61.0
Spergon	0.33	8.8	18.6	27.4	6.4	21.0	27.4
Zinc oxide AAZ	0.99	17.4	19.2	36.6	12.8	23.8	36.6
Cuprocide	2.00	37.6	16.8	54.4	28.8	25.6	54.4
No treatment	.....	9.8	10.8	20.6	6.2	14.4	20.6
Least Mean Sign. Diff.							
5 per cent	.....	14.07	9.69	9.10	13.11	9.93	9.11
1 per cent	.....	18.76	12.92	12.14	17.47	13.24	12.14

provided fair protection against pre-emergence damping-off, but permitted excessive post-emergence damping-off. Spergon provided little protection against damping-off at any stage.

In the October experiment (Table 4) Thiosan (a wettable preparation containing 50 per cent tetramethyl thiuramdisulphide) was tested and found to be comparable at equal rates of active material to the 80 per cent

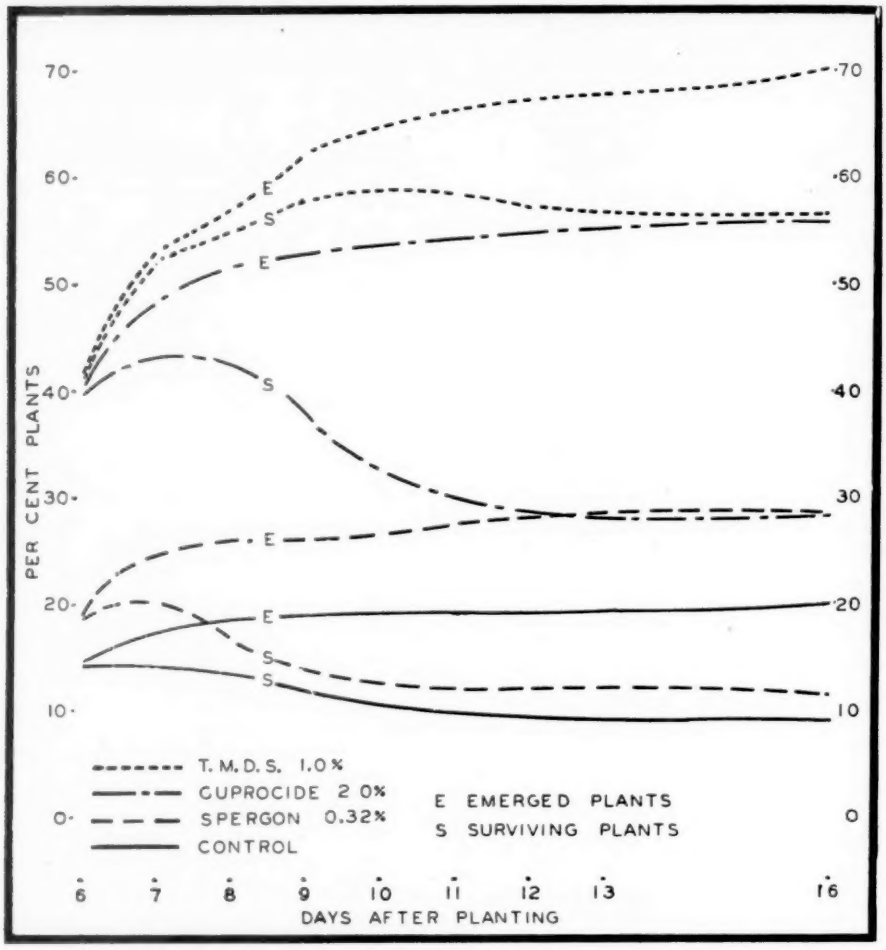

FIG. 1 EMERGENCE AND SURVIVAL OF SPINACH SEEDLINGS AS A FUNCTION OF TIME AND TREATMENT,- COMBINED DATA — AVG. 1000 SEEDS

form (T.M.D.S.) previously used. In this test Cuprocide was more effective than in the September test (Cuprocide plots in both tests were planted from a lot of seeds treated in late August); hence, only the highest dosages of T.M.D.S. and Fermate gave significantly higher stands than Cuprocide. Spergon and zinc oxide failed to protect spinach adequately in this test.

The data from treatments common to both tests were combined, the

homogeneity test described by Hayes and Immer (15) showing that the variances of these two tests are homogeneous. These combined data show that, at similar dosages, T.M.D.S. resulted in significantly more surviving plants than did treatment with Fermate, though the total emergence was approximately equal. Reduction of the dosage with both these materials resulted in reduced control of both pre-emergence and post-emergence damping-off. Cuprocide was significantly poorer than T.M.D.S. and Fermate for plant survival, for post-emergence control, and, with the exception of the lowest

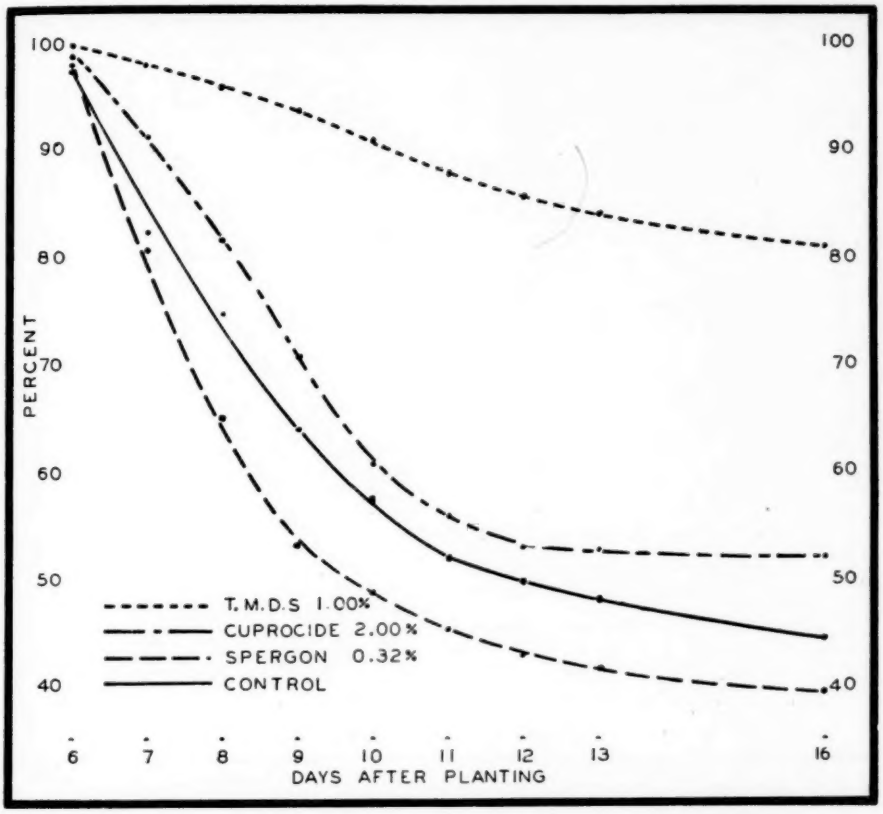

FIG. 2 – PER CENT SURVIVAL OF EMERGED SPINACH SEEDLINGS AS A FUNCTION OF TIME AND TREATMENT

dosages of these materials, for pre-emergence control of damping-off. Spergon was barely significant over no treatment in pre-emergence control and was very ineffective in the control of post-emergence damping-off.

The daily progress of seedling emergence and damping-off, as shown by plant survival, with the combined data of a few selected treatments, have been charted in figure 1. This shows the effect of T.M.D.S. in the protection of the late emerging seedlings, also the comparatively low mortality of the emerged seedlings under conditions highly conducive to damping-off. Noteworthy is the failure of Cuprocide to control post-emergence damping-off as

shown by the early and heavy loss of plants. The ineffectiveness of Spergon is shown by the similarity in slope of the curves for this material and the controls.

To further examine the effectiveness of protection against post-emergence damping-off afforded by different treatment materials, the percentage of survival of those plants which emerged is charted in figure 2. This chart shows that post-emergence damping-off with Cuprocide, Spergon, and with no treatment is early and rapid and follows a very similar pattern, as con-

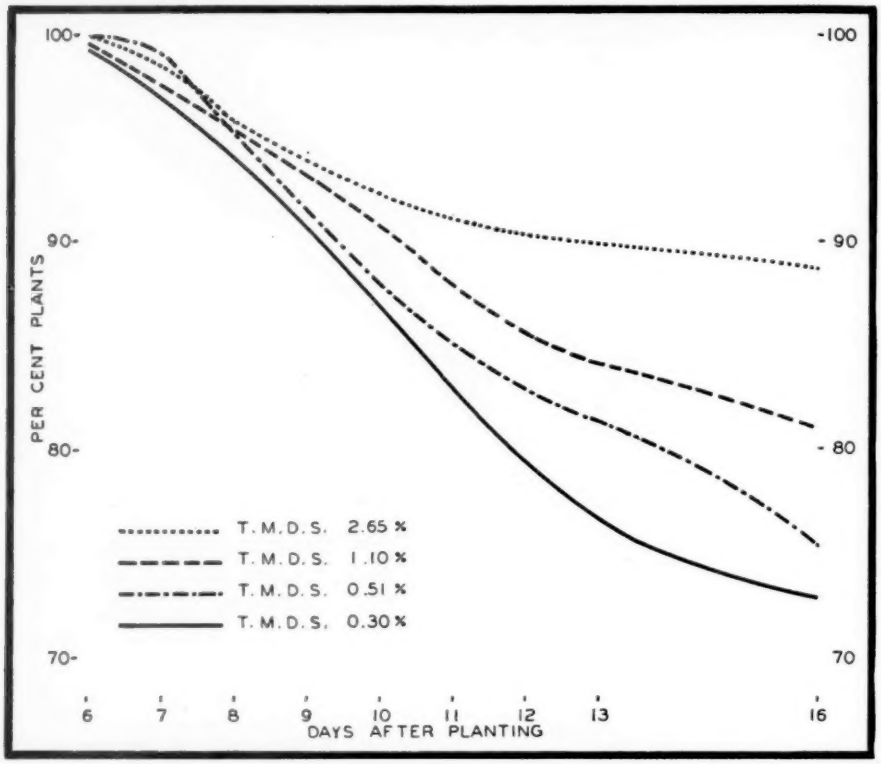

FIG. 3.- PER CENT SURVIVAL OF EMERGED SPINACH SEEDLINGS AS A FUNCTION OF TIME AND DOSAGE OF TETRAMETHYL THIURAMDISULPHIDE

trasted with the delayed pattern of post-emergence damping-off with T.M.D.S. at one per cent. It should be noted that the data in this figure were obtained by the formula *number surviving* $\times$ *100/number which had emerged.* Thus, 100 surviving of a total of 200 emerged plants would give the same survival percentage as 250 surviving from a total emergence of 500 plants, even though the latter represents a much better stand of plants from the 1000 seeds planted.

Data from these two experiments are available on the effect of different dosages of tetramethyl thiuramdisulphide on the control of post-emergence damping-off. The percentage survival of emerged plants, as charted in fig-

ure 3, shows that the control of post-emergence damping-off increased as the treatment rate was increased.

TETRACHLORORESORCINOL AND COPPER TRICHLORPHENATE AS SEED PROTECTANTS ON SPINACH

Tetrachlororesorcinol and C–119 (a preparation containing 20 per cent copper trichlorphenate) at two dosages each were compared with Thiosan at 1.5 per cent in one test, table 5 and figure 4.

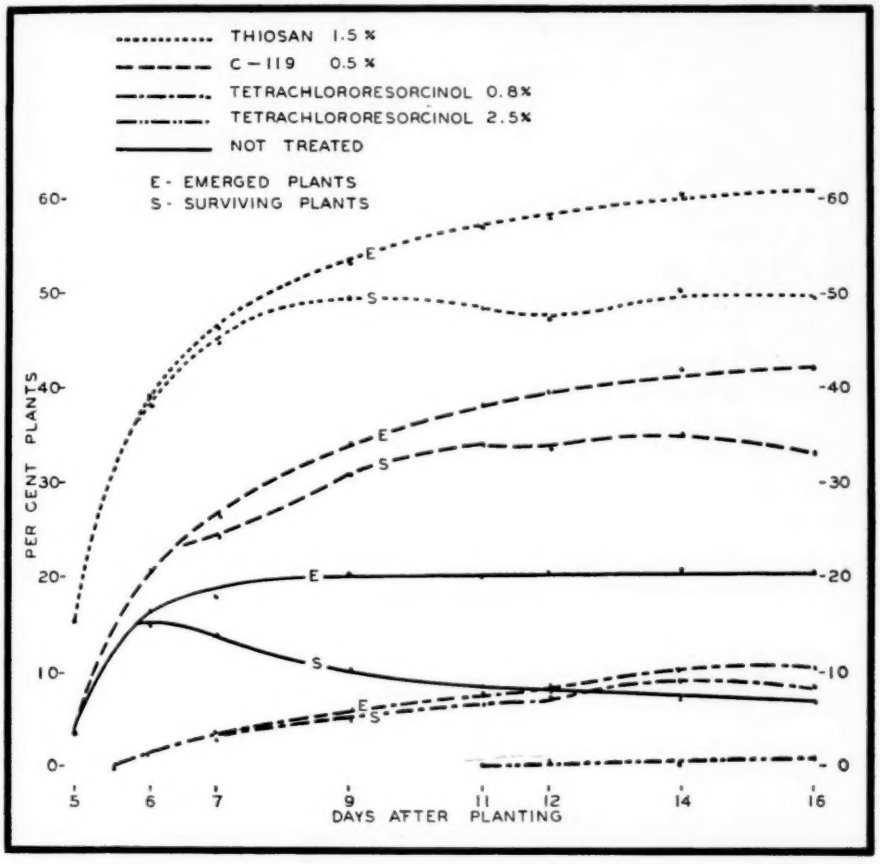

FIG. 4.– EMERGENCE AND SURVIVAL OF SPINACH SEEDLINGS AS A FUNCTION OF TIME AND TREATMENT WITH TETRACHLORORESORCINOL AND COPPER TRICHLORPHENATE

These data show that tetrachlororesorcinol at 2.5 per cent dosage permitted the emergence of but three plants from 500 seeds, but that a 12.2 per cent emergence occurred with a dosage of 0.8 per cent. While this experiment was not duplicated in partially sterilized soil, the data show clearly that the low emergence was due to injury and not to lack of protection. This injury was further demonstrated by the delayed emergence (Fig. 4). At the lower dosage of 0.8 per cent, 23 per cent of those plants which had

TABLE 5.—*Copper trichlorphenate and tetrachlororesorcinol as seed protectants on spinach planted Oct. 23, 1942. Five replications of 100 seeds each*

Material	Dosage (Per cent)	Percentage of plants 18 days after planting		
		Surviving	Damped-off	Emerged
Thiosan	1.5	48.4	12.6	61.0
C–119	1.5	12.0	0.8	12.8
Do	0.55	32.8	9.8	42.6
Tetrachlororesorcinol	2.5	0.6	0.0	0.6
Do	0.8	9.4	2.8	12.2
No treatment	.....	8.4	13.4	21.8

emerged had damped-off within 18 days after planting. This result suggests that protection against damping-off would not be satisfactory at dosages sufficiently low to be non-injurious.

C–119 at 1.5 per cent also proved to be excessively injurious, giving both delayed and low emergence. However, only 6.3 per cent of those plants which emerged damped-off within 18 days, thus showing a high order of protection against post-emergence damping-off. At the lower dosage of 0.5 per cent, while the emergence was considerably lower than with Thiosan, post-emergence protection was excellent. Further trials of these materials were not made, inasmuch as they appeared unsafe on spinach.

SILVER SALTS AS SEED PROTECTANTS ON SPINACH

The promising results obtained with silver carbonate in the preliminary trials prompted further testing of this material, together with silver chro-

TABLE 6.—*Silver salts compared with other seed protectants on spinach, planted Dec. 28, 1942. Five replications of 50 seeds each*

Material	Dosage (Per cent)	Percentage of plants 16 days after planting			
		Infested soil			Partially sterilized soil
		Surviving	Damped-off	Emerged	Emerged
Silver carbonate	3.82	10.4	38.8	49.2	76.0
Do	0.99	9.6	44.8	54.4	78.8
Do	0.31	9.6	26.8	36.4	79.2
Silver chromate	4.04	52.0	26.8	78.7	76.0
Do	0.82	30.8	39.2	70.0	81.6
Silver oxide	2.12	10.0	55.2	65.2	73.6
Do	0.40	13.6	37.2	50.8	76.0
Sil. orthophosphate	2.76	18.0	41.2	59.2	77.2
Do	0.27	4.8	22.4	27.2	73.2
T.M.D.S.	0.99	55.2	25.2	80.4	88.0
Thiosan	1.48	58.4	22.4	80.8	82.4
Cuprocide	2.00	26.8	37.6	64.4	82.0
Iron sulfate · $1.5H_2O$	2.20	27.4	32.4	58.8	78.4
No treatment	.....	8.4	13.2	21.6	76.4
Least Mean Sign. Diff.					
5 per cent	.....	15.9	14.2	14.1	10.1
1 per cent	.....	21.1	18.9	18.8	.....

mate, silver oxide, and silver orthophosphate. These were pulverized in an agate mortar to pass through a 100-mesh screen to insure small particle size. By such means it was possible to apply dosages sufficient to detect any tendency toward seedling injury. To explore further any tendency to cause injury, this experiment was duplicated in soil from the lot discussed under "Methods." However, in this duplicate test the soil was partially sterilized by the formaldehyde treatment method of Guterman and Massey (13).

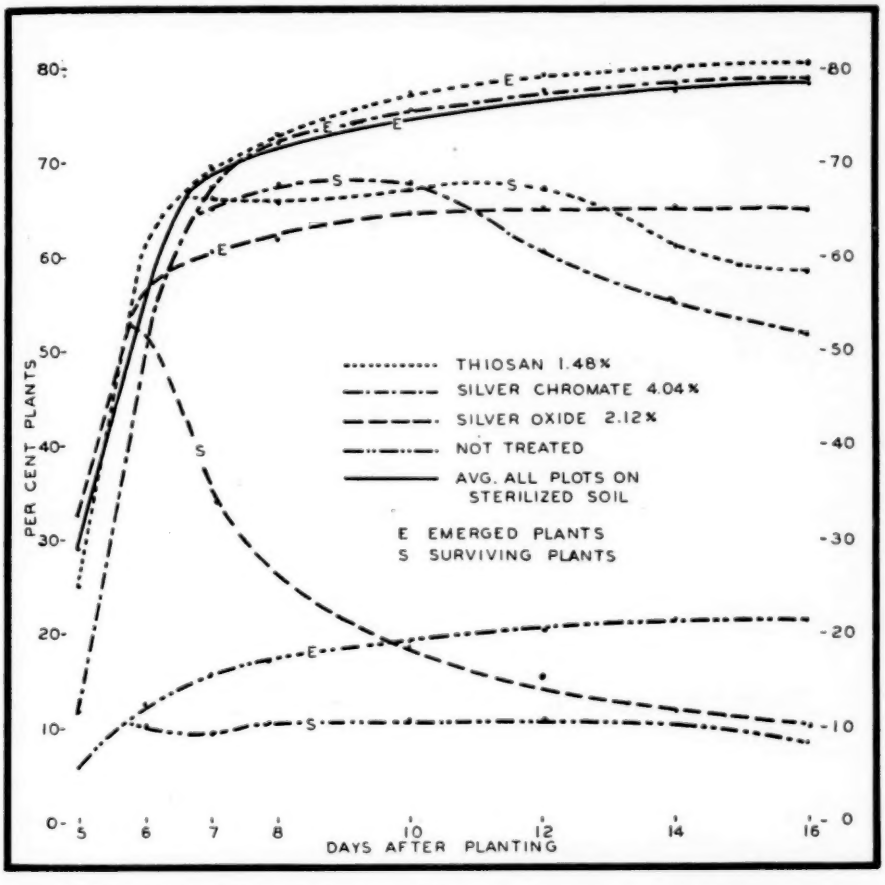

FIG. 5.—EMERGENCE AND SURVIVAL OF SPINACH SEEDLINGS AS A FUNCTION OF TIME AND TREATMENT — 250 SEEDS PER TREATMENT.

Table 6 shows that none of these materials significantly reduced the plant stand at 16 days when planted in partially sterilized soil; however, data taken during the period of most rapid emergence suggest that high dosages of silver chromate delayed emergence slightly. Silver chromate at 4.0 per cent dosage gave sufficient protection to result in a final stand comparable to that obtained with T.M.D.S.; at 0.8 per cent it was much less effective, being nonsignificantly better than Cuprocide in any respect. Silver carbonate, silver orthophosphate, and silver oxide were ineffective in protecting the

emerged plants against post-emergence damping-off though each, with the exception of silver orthophosphate at 0.2 per cent dosage, offered a considerable degree of protection through the pre-emergence period. The iron sulphate tested was surprisingly effective in this test, being as effective as Cuprocide. A further comparison between T.M.D.S. and Thiosan at approximately equal dosages of tetramethyl thiuramdisulphide showed these materials to be of equal effectiveness and to be significantly better than any other materials in this test, with the exception of silver chromate at 4.0 per cent dosage. With these three materials, the number of plants which emerged in heavily infested soil approached closely the number which emerged in

TABLE 7.—*Seed protectants on Big Boston lettuce grown in the greenhouse*

Material	Dosage (Per cent)	Five replications of 100 seeds, planted Sept. 26, 1942 Percentage of plants at 15 days			Four replications of 50 seeds, planted Nov. 21, 1942 Percentage of plants at 16 days		
		Surviving	Damped-off	Emerged	Surviving	Damped-off	Emerged
T.M.D.S.	4.18	69.2	1.8	71.0	60.5	4.5	65.0
Do	0.99	80.2	1.2	81.4	70.5	6.5	77.0
Do	0.50	82.6	2.4	85.0	59.5	16.0	75.5
Do	0.29	79.0	2.8	81.8	50.5	18.5	69.0
Do	0.15	78.0	3.2	81.2	64.5	8.0	72.5
Thiosan	3.25	.....	.....	.....	56.5	4.0	60.5
Do	1.65	81.0	1.0	82.0	58.0	10.0	68.0
Do	0.75	.....	.....	.....	49.0	16.0	65.0
Fermate	11.96	75.2	0.8	76.0	57.5	4.5	62.0
Do	1.00	84.6	2.2	86.8	61.0	14.5	75.5
Do	0.56	77.0	3.6	80.6	55.5	12.0	67.5
Do	0.38	73.8	4.2	78.0	60.5	13.0	73.5
Do	0.15	66.8	3.6	70.4	46.5	12.0	58.5
Spergon	0.28	76.0	2.4	78.4	.....	.....	.....
Cuprocide	1.82	93.0	2.2	95.2	82.5	3.5	86.0
No treatment	.....	35.4	4.0	39.4	26.5	5.5	32.0
Least Mean Sign. Diff.							
5 per cent	.....	7.1	Not	6.0	16.1	8.6	13.3
1 per cent	.....	9.4	sign.	8.0	21.5	11.5	17.7

partially sterilized soil, thus showing their very great effectiveness in preventing pre-emergence damping-off.

The transient nature of the protection offered by most of the silver salts is well illustrated by silver oxide at 2.1 per cent dosage, as plotted in figure 5. With this material a stand of 52.4 per cent was reached on the sixth day, but this stand was reduced by post-emergence damping-off to 25.2 per cent by the eighth day and to 10 per cent by the sixteenth day. That this material afforded a considerable amount of pre-emergence protection is shown by the total emergence of 65.2 per cent. Silver chromate, on the other hand, when used at 4.0 per cent dosage, gave a high degree of protection throughout this experiment, though causing a slight lag in emergence on the fifth and sixth days. The excellent protection against pre-emergence damping-off provided by silver chromate at 4.0 per cent and by T.M.D.S. and Thiosan

is evidenced by the fact that emergence with these materials in infested soil was equal to the average emergence with all treatments on partially sterilized soil (shown as the solid line in figure 5).

SEED PROTECTANTS ON LETTUCE

Two trials were conducted on Big Boston lettuce, the data from which are presented in table 7. In one of these 100 seeds were planted in each of

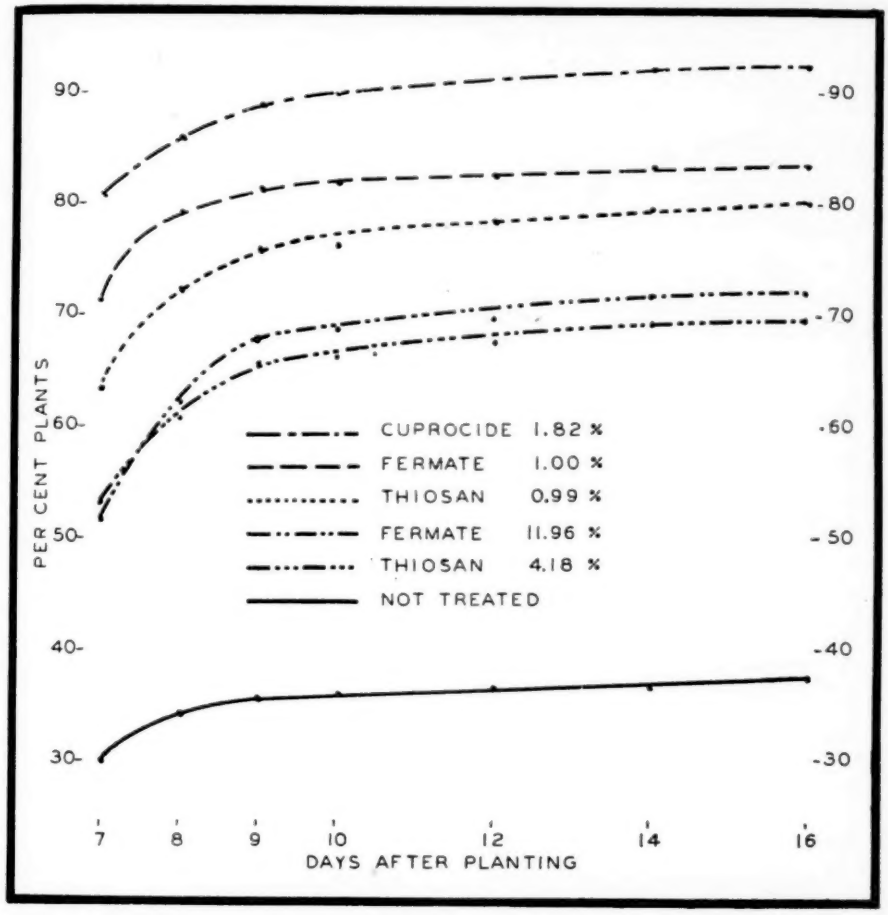

FIG. 6.-EMERGENCE OF LETTUCE SEEDLINGS AS A FUNCTION OF TIME AND TREATMENT — 700 SEEDS PER TREATMENT.

five replications; in the other, 50 seeds were planted in each of four replications.

In these experiments all materials tested gave very significant increases in stand over the untreated controls. Cuprocide, at 1.82 per cent dosage, gave excellent results in both trials, being significantly better than most of the other treatments. With T.M.D.S. and with Fermate, the optimum dosage

appeared to be near 0.5 to 1.0 per cent. The tendency towards greater incidence of post-emergence damping-off at the lower dosages of these materials suggests that the lower emergence here probably was due to failure to control adequately pre-emergence damping-off. The reduced stands with the highest dosage of these materials is believed to have been due to injury to the seeds or seedlings. The plants in the highest dosage plots were dwarfed slightly throughout the experiments, supporting the probability that injury was a factor in this case.

The data on plant emergence from the combined tests are presented in figure 6 and serve to illustrate the greater benefits obtained from the use of Cuprocide. This figure also illustrates the lower stands when more than the optimum dosage was applied with both T.M.D.S. and with Fermate.

SEED PROTECTANTS ON CUCUMBER

Table 8 presents the results obtained from the use of various protectants on cucumber seed. All treatments tested gave highly significant increases

TABLE 8.—*Effect of seed protectants on cucumber planted Feb. 24, 1943. Six replications of 40 seeds each*

Material	Dosage (Per cent)	Percentage of plants 17 days after planting		
		Surviving	Damped-off	Emerged
Thiosan	0.25	79.2	7.1	86.3
Do	0.12	77.1	10.8	87.9
Do	0.05	70.0	13.8	83.8
Fermate	0.20	74.2	12.5	86.7
Do	0.10	78.8	7.5	86.3
Do	0.05	76.3	10.0	86.3
Spergon	0.23	60.4	19.2	79.6
Do	0.13	62.5	20.4	82.9
$FeSO_4 \cdot 1.5H_2O$	0.63	51.7	18.8	70.4
Semesan	0.22	69.6	17.9	87.5
Cuprocide	0.31	63.8	19.6	83.3
No treatment	.....	5.8	12.9	18.8
Least Mean Sign. Diff.				
5 per cent	.....	16.1	Not	9.1
1 per cent	.....	21.4	sign.	12.1

over the untreated control in emergence and in the final stand at 17 days after planting. Among the treatments only the iron sulphate was significantly less effective than any other material at the 1 per cent level. An apparent trend toward lower amounts of post-emergence damping-off with Thiosan and with Fermate, particularly at the higher dosages, proved to be non-significant upon statistical examination. The daily progress of emergence and damping-off for a few materials is charted in figure 7. Here the marked increase in stand from the use of any of these treatments is apparent by reference to the untreated control.

CABBAGE SEED TREATMENT

Since Cuprocide repeatedly has been shown to be injurious to members of the Brassicaceae, a test of some of the newer materials was conducted on

cabbage. The results of a greenhouse test are shown in table 9 and in figure 8.

In this test, treatment with Thiosan, Fermate, and zinc oxide yielded approximately equal emergence of plants. All dosages of Thiosan and

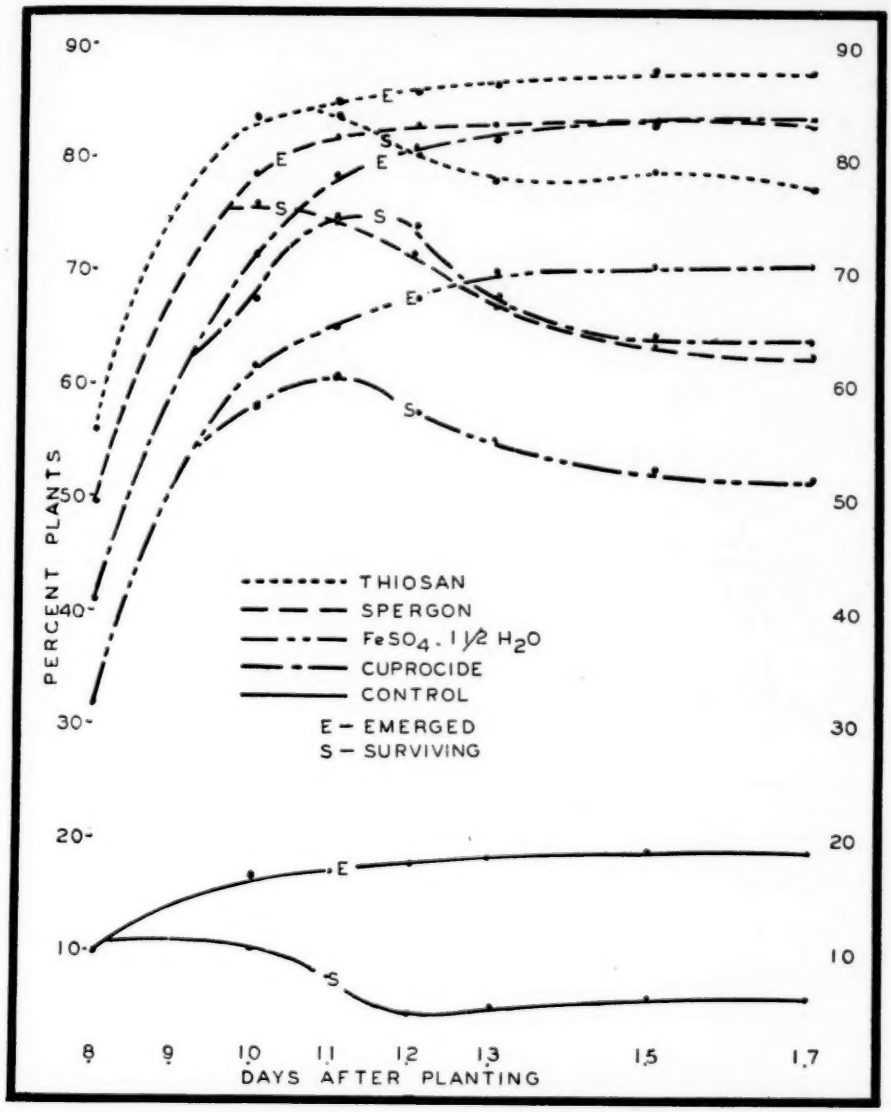

FIG. 7.- EMERGENCE AND SURVIVAL OF CUCUMBER SEEDLINGS AS A FUNCTION OF TIME AND TREATMENT — 240 SEEDS PER TREATMENT

T.M.D.S. resulted in an average emergence of 79.3 per cent, while all dosages of Fermate averaged 79.2 per cent. In this experiment post-emergence damping-off was so slight that no conclusions may be drawn on this phase of protection.

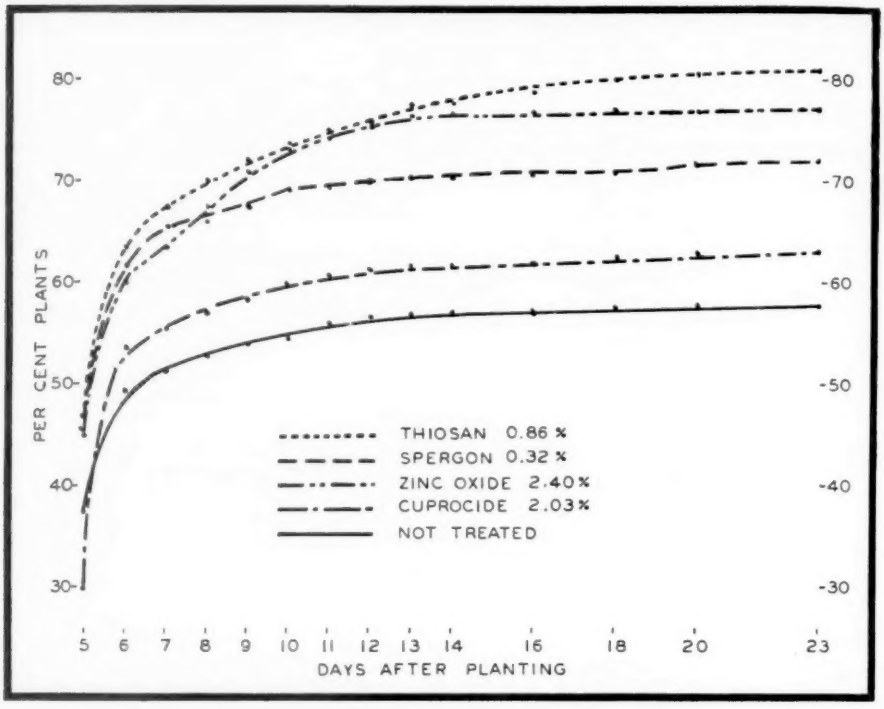

FIG. 8.-EMERGENCE OF CABBAGE SEEDLINGS AS A FUNCTION OF TIME AND TREATMENT — 300 SEEDS PER TREATMENT.

TABLE 9.—*Effect of seed protectants on cabbage planted Nov. 17, 1942. Six replications of 50 seeds each*

Materials	Dosage (Per cent)	Percentage of plants 23 days after planting		
		Surviving	Damped-off	Emerged
T.M.D.S.	0.56	83.3	1.7	85.0
Thiosan	3.11	77.3	1.0	78.3
Do	1.48	77.0	2.0	79.0
Do	0.86	78.3	2.3	80.7
Do	0.45	70.0	5.3	75.3
Do	0.23	73.3	4.3	77.7
Fermate	3.21	84.3	0.3	84.7
Do	0.95	79.3	1.0	80.3
Do	0.52	79.0	3.3	82.7
Do	0.27	72.7	2.3	75.0
Do	0.15	72.7	1.0	73.7
Spergon	0.32	71.0	1.0	72.0
Zinc oxide	2.40	77.0	0.0	77.0
Semesan	0.42	71.0	1.7	72.7
Cuprocide	2.03	59.0	4.0	63.0
No treatment	.....	55.3	2.0	57.3
Least Mean Sign. Diff.				
5 per cent	.....	7.0	Not	6.3
1 per cent	.....	9.3	sign.	8.4

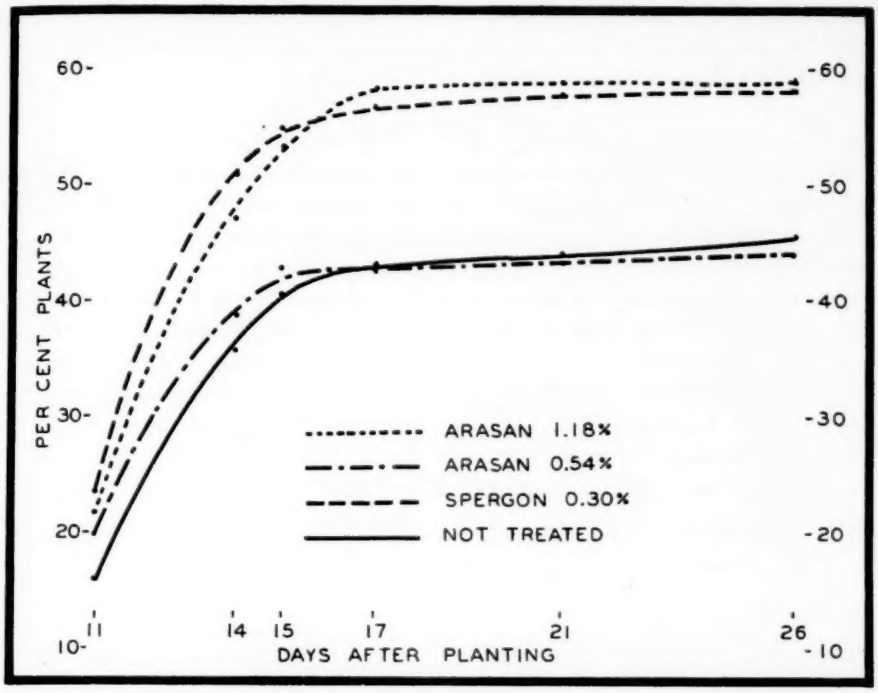

FIG. 9.- EMERGENCE OF CARROT SEEDLINGS AS A FUNCTION OF TIME AND TREATMENT — 250 SEEDS PER TREATMENT

Treatment with Spergon and with Semesan, at recommended dosages, resulted in slightly lower emergence than did treatment with Thiosan, Fermate, and zinc oxide. The daily progress of emergence with Spergon, figure 8, was very similar to that of Semesan (not shown).

TABLE 10.—*Effect of seed protectants on carrot planted Apr. 6, 1943. Five replications of 50 seeds each*

Materials	Dosage (Per cent)	Percentage of plants 26 days after planting		
		Surviving	Damped-off	Emerged
Arasan	2.24	58.0	0.8	58.8
Do	1.18	58.0	1.2	59.2
Do	0.54	42.0	2.0	44.0
Do	0.26	42.4	1.6	44.0
Fermate	4.75	55.6	0.0	55.6
Do	1.10	57.2	1.2	58.4
Do	0.52	47.6	1.6	49.2
Do	0.27	52.0	1.2	53.2
Spergon	0.30	55.6	2.4	58.0
Semesan	0.42	56.0	4.0	60.0
Cuprocide	2.28	52.8	1.6	54.4
No treatment	.....	40.8	2.4	45.6
Least Mean Sign. Diff.				
5 per cent	.....	7.76	Not	7.6
1 per cent	.....	10.38	sign.	10.2

Cuprocide, in this test, resulted in emergence significantly lower than that in any other treatment series and the emergence was not significantly higher than that with no treatment.

CARROT SEED TREATMENT

A single experiment was conducted on the treatment of carrot seed. For this trial Arasan was available and, since this appeared to be the form in which tetramethyl thiuramdisulphide would be marketed as a seed treatment material, it was substituted for T.M.D.S. and Thiosan.

In this experiment, only Arasan at 0.5 per cent or lower dosage failed to give appreciable increases in plant emergence and survival (Table 10 and Fig. 9). Arasan and Fermate at dosages of 1.1 per cent or higher, Spergon at 0.3 per cent, Semesan at 0.42 per cent, and Cuprocide at 2.3 per cent all offered such protection that both the emergence and the survival of plants were significantly better than those of the controls.

The incidence of post-emergence damping-off was low in this experiment and no significant differences in its control were obtained.

TOMATO SEED TREATMENT

Arasan, Thiosan, Fermate, Spergon, Cuprocide, and talc were tested on tomato seed in a single trial. Here dosage rates were achieved by dilution of the treatment material. The data from this test are not presented since an excellent stand of plants was obtained regardless of treatment, the controls being nonsignificantly poorer than any of the protective treatments.

DISCUSSION

In these studies the practice of making frequent counts of healthy and of damped-off seedlings has permitted graphic portrayal of the response to the use of various seed protectants. Spinach, being highly susceptible to damping-off both before and after emergence from the soil, was found to be especially suited to the testing of new materials.

Under conditions of temperature and moisture favorable to attack by *Pythium ultimum*, Arasan and the other tetramethyl thiuramdisulphide mixtures proved to be very effective protectants against pre-emergence damping-off. They were more effective than the already widely used Cuprocide, Spergon, and, where tested, Semesan. However, the most striking effect of Arasan and of related materials has been found in the high degree of protection they have offered against post-emergence damping-off. This continued protective value promises great effectiveness where seed germination is long-delayed by unfavorable weather conditions. The effect of Arasan in protecting against such unfavorable weather conditions is directly proportional to the dosage applied and it, therefore, is important that seeds not only be treated but that adequate dosages be used.

Fermate approached Arasan closely in its effectiveness against both pre-emergence and post-emergence damping-off. However, the black color of

this material, as compared with the pink color of Arasan, offers a disadvantage where seeds are not to be machine planted. In hand seeding it is very difficult to determine the spacing with Fermate-treated seeds due to their inconspicuous color.

Cuprocide was reasonably effective in preventing pre-emergence damping-off, but this protection lasted for a very few days. After emergence, plants from Cuprocide-treated seeds frequently were as subject to post-emergence damping-off as were seedlings from untreated seeds.

Spergon, when used on spinach at dosages recommended by the manufacturer, was inadequate under the conditions of these tests. Its low effectiveness on spinach is in agreement with the findings of other workers.

The use of duplicate plantings in partially sterilized soil offers a method of differentiating between low emergence due to chemical injury to the seedling and low emergence due to poor protection against pre-emergence damping-off.

SUMMARY

1. A study was made of the value of numerous chemicals in combating damping-off when dusted on vegetable seeds. Particular attention was given to refinements in technique so that the relative values of the materials and dosages could be compared more precisely. Plant counts at frequent intervals permitted study of the influence of the protectant material on the control of damping-off at different stages in the development of the seedling.

2. Arasan and other mixtures of tetramethyl thiuramdisulphide were the most effective of the seed protectants tested, except with lettuce. Their protective influence continued into the seedling stage to a greater extent than occurred with the previously used seed protectants.

3. The protective value of Arasan and of related materials was in direct proportion to the dosage applied.

4. Fermate approached Arasan in effectiveness as a seed protectant.

5. The protective effect of Cuprocide was dissipated within a few days under the conditions of these tests.

6. Silver chromate at very high dosages offered good protection; silver oxide was protective for a very few days.

7. Spergon was low in protective value.

8. Seed treatment with Arasan was successful with spinach, cucumber, carrot, cabbage, and tomato. On lettuce both Arasan and Fermate were less beneficial than Cuprocide, the data indicating that Arasan and Fermate were injurious to lettuce.

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A STUDY OF THE OCCURRENCE OF PHOMOPSIS AND OF DIPLODIA ROTS IN FLORIDA ORANGES UNDER VARIOUS CONDITIONS AND TREATMENTS

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Although stem-end rot of oranges is usually considered as one disease, it is well known that two causal organisms, *Phomopsis citri* Faw. (*Diaporthe citri* (Faw.) Wolf) and *Diplodia natalensis* Evans (*Physalospora rhodina* (B. & C.) Cke.), are involved. Either organism alone is capable of producing the characteristic rot and because of the similarity of symptoms it is nearly impossible to tell by macroscopic examination which organism is causing stem-end rot in an individual fruit.

To gain insight into the action of each of these fungi, observations were taken and isolations made at weekly intervals from all the fruit suspected of having rot among 850 separate samples of 50 fruits each. These samples were taken from approximately 125 different tests which were set up to study various phases in stem-end-rot control. These tests took into consideration such factors as appear in (1) the groves, (2) picking, (3) packinghouse operations, and (4) storage. Most of the investigations dealt with here are related to the third group. The samples in the various similar tests were so arranged that the data obtained could be treated statistically by the analysis of variance method. The 5 per cent level of probability was chosen as the criterion of significant differences.

EFFECTS OF GASSING, DEBUTTONING BY VARIOUS METHODS, AND BORAX TREATMENTS

In an experiment, consisting of 16 tests obtained from 4 replications of each of the varieties, Hamlin, Parson Brown, Pineapple, and Valencia, throughout the picking season, reported by Hopkins, Loucks, and Stearns,[3] a study was made of the effect of various treatments, applied both separately and in combination, on stem-end rot. The single treatments were: gassing with ethylene, for 48 hours at 85° F. and 90 per cent relative humidity, at a concentration comparable to that used in commercial gassing chambers for degreening oranges; removing buttons after 48 hr. gassing; soaking in a 5 per cent solution of borax for 5 min. at 110° F. and not rinsing off except where especially noted. Borax was applied before and/or after gassing. Only those combinations that appeared likely to be effective were tried. *Phomopsis* and *Diplodia* were isolated from decayed specimens of the 50 fruits used for each sample: the percentages of each fungus that

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[3] Hopkins, E. F., K. W. Loucks, and C. R. Stearns, Jr. A study of certain methods for the control of stem-end rot and blue mold in oranges. Proc. Fla. State Hort. Soc. 57: 87–98. 1944.

developed within three weeks after treatment are indicated in figures 1 and 2, respectively.

The least difference necessary for significance among the results for *Phomopsis* is 5.2 per cent, and for *Diplodia* 8.6 per cent. In the figures all treatments designated by the same letter are of equal importance so far as the development of the fungi is concerned. For instance, the difference between the average percentage of *Phomopsis* present in 3 weeks from Treatment 11 was not significantly different from that with Treatment 1, but it was significantly different from Treatment 12. Likewise, Treatment 12 was not significantly different from Treatment 8 but was from Treatments 10 and 4. As there was a possibility that longer storage might change the picture, the data for 5 weeks were similarly analyzed, but the results showed that the general trend was still the same as at 3 weeks.

Because 3 weeks is considered to be the nearest representation of the average time which elapses from picking a commercial fruit until it is consumed, the results after 3 weeks are the ones given the most consideration and reported in this paper.

Debuttoning effectively controlled *Phomopsis* (Fig. 1). If the buttons were not removed, borax, alone or used in the various combinations, was no better than the ungassed check (Treatment 8). When used with gassed fruit, either before, or before and after, the ethylene treatment, borax reduced the amount of *Phomopsis*. With only the gas treatment, more *Phomopsis* developed than in the checks. When the buttons were removed from borax-treated fruit, the effect of gassing was overcome. Borax applied before gassing fruit with buttons left on was not better than borax applied after gassing for controlling *Phomopsis*.

Debuttoning also was very effective in reducing *Diplodia* (Fig. 2). Borax applied before, or before and after, gassing was effective in reducing *Diplodia* if the buttons were left on. If the buttons were removed the effect of borax, if any, was masked by the good control attendant upon button removal even though the fruit was gassed. Gassing fruit or leaving the buttons on increased the amount of *Diplodia*.

Another experiment with 4 replications from the varieties Valencia and Pineapple was set up to test the various methods of partially or wholly debuttoning oranges for stem-end-rot control. These were also combined with the borax treatment before and after gassing, and the borax treatments were either left on or washed off. Seventeen predetermined treatment combinations, as shown in figures 3 and 4, were used. The various button treatments were as follows:

(a) Button left intact by clipping the fruit.

(b) Calyx removed by pulling fruit or by a drill-press reamer.

(c) Button completely removed by hand after gassing fruit for 48 hours.

Other treatments were the same as in the previous experiment except that with some of the combinations with borax treatment the borax was washed off after the fruit had soaked for 5 minutes.

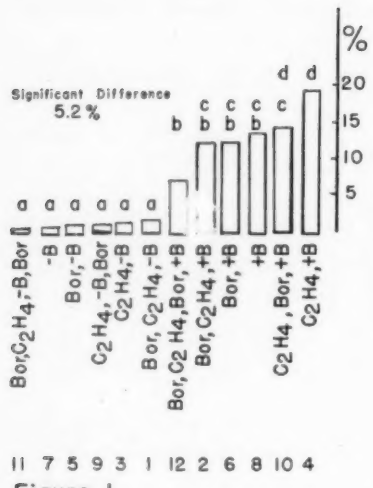

Figure 1

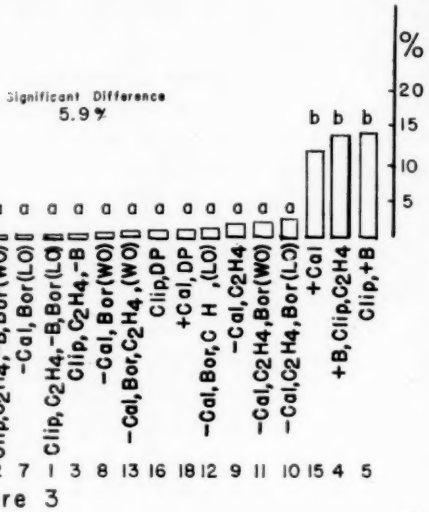

Figure 3

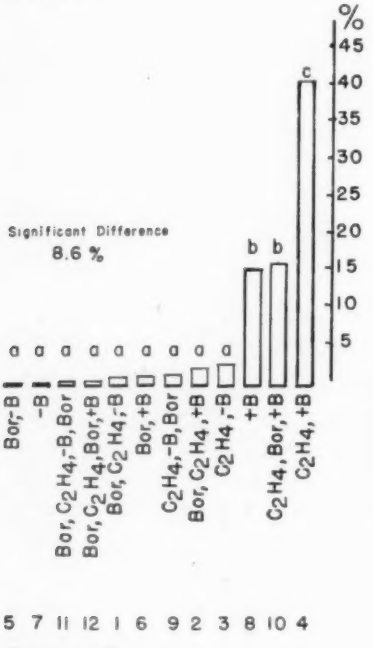

Figure 2

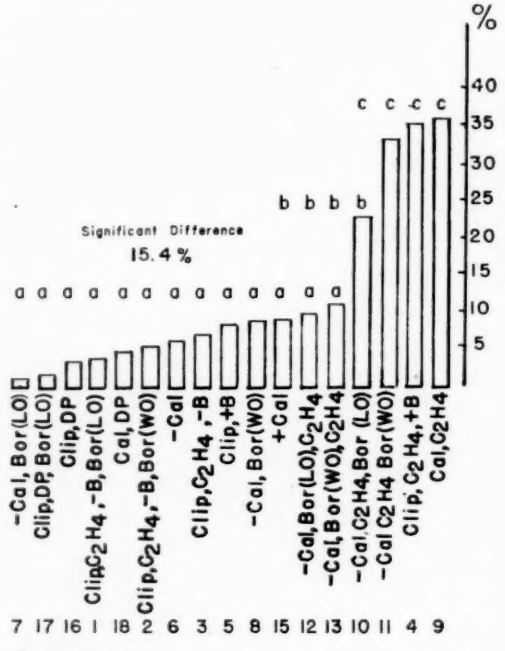

Figure 4

FIG. 1 THROUGH 4. Development of *Phomopsis* (Fig. 1 and 3) and of *Diplodia* (Fig. 2 and 4) during 3 weeks in storage after various fruit treatments: Borax, 5 per cent solution, at 110° F. for 5 minutes (Bor) and solution left on fruit (L O) or washed off (W O); Gassing with ethylene for 48 hr. at 85° F. and 90 per cent relative humidity (C_2H_4); All buttons left on fruit (+B) or removed (−B); Calyx left intact (+Cal) or removed (−Cal) when fruit was picked by pulling or calyx removed by reamer in drill press (DP); Fruit picked by clipping (Clip).

Treatments designated by the same letter are of equal value.

The data for the 3-week periods were used to obtain the information presented in figure 3 where the various treatments are arranged in their order of rank in controlling *Phomopsis*. The least difference necessary for significance between treatments at the 5 per cent level is 5.9 per cent. Treatments that do not show this difference are designated by assigning to them the same letter. The striking result is that no matter how the calyx is removed, whether by pulling or by removing the entire button, or by reaming, *Phomopsis* was reduced very significantly regardless of what other treatment or treatments were combined with the debuttoning. *Phomopsis* was not increased by gassing. Pulled fruit with the calyx left on was not different from clipped fruit (calyx on). Among the various treatments in which borax was used there was not sufficient difference to determine the effect of borax, and nothing was deducible concerning borax left on as against borax washed off.

In figure 4 is presented the same information about *Diplodia*. Here the corresponding least difference between the averages of the percentage of *Diplodia* necessary for significance is 15.4 per cent. The lack of differences is designated by letters in the same manner as in figure 3.

Most striking is the fact that fruit that lost its calyx when pulled from the tree was very susceptible to *Diplodia* when subsequently gassed (Fig. 4). Ethylene applied to fruit that had its calyx intact also was very active in increasing *Diplodia*. This effect of gassing upon *Diplodia* was not overcome by removing the calyx by pulling the fruit, even if borax was applied after the gassing and left on. It was successfully counteracted only by removing buttons or by removing the buttons and then treating the fruit with borax (even though it was washed off) after gassing. Calyx removal by any of the methods used had no effect on *Diplodia*.

No matter how the fruit was otherwise treated, gassing did not significantly increase *Phomopsis* but with few exceptions did very substantially increase *Diplodia*. When the fruit was debuttoned after gassing, but not otherwise treated, the effect of gassing was overcome. Some of these same results have been reported by Brooks,[4] Voorhees,[5] and others.

CORRELATION BETWEEN OCCURRENCE OF PHOMOPSIS AND DIPLODIA

From 170 samples of 50 fruit each, which were picked under similar conditions and used as checks for that many separate experiments, data were obtained concerning the development of *Phomopsis* and of *Diplodia* to determine their interrelationship. Storage conditions were not comparable for all samples, but aside from that the fruit was all treated alike. The fruit was picked by clipping, randomized into samples, and immediately placed in storage. The varieties Hamlin, Parson Brown, Pineapple, and Valencia were represented.

[4] Brooks, Charles. Stem-end rot of oranges and factors affecting its control. Jour. Agr. Res. [U. S.] **68**: 363–381. 1944.

[5] Voorhees, R. K. Investigations of melanose and stem-end rot of citrus fruit. Ann. Rept. Fla. Agr. Exp. Sta. p. 188–191. 1944.

TABLE 1.—*Regression and correlation coefficients, obtained from the data from 4 varieties of oranges, showing the lack of interrelationship between Phomopsis and Diplodia*

Variety	Regression Coefficient	Correlation Coefficient
Hamlin	0.321	0.212
Parson Brown	0.143	0.143
Pineapple	0.041	0.038
Valencia	0.515	0.347[a]

[a] Only data from the Valencia oranges showed significant correlation between the occurrence of *Phomopsis* and of *Diplodia*.

When the data from all varieties were combined to form a correlation table to test relationship between occurrence of *Phomopsis* and *Diplodia* based on results after 3 weeks in storage, the linear regression line had a positive trend but the points were too scattered for the correlation to be significant.

The linear regression was applied to data from each variety separately and the coefficients of regression and correlation coefficients are shown in table 1. These data indicate that whatever combination of factors produced an increase in one of the causal organisms did not cause a decrease in the other and there is no proof that the converse is true. Apparently there is no interrelationship between the two organisms.

HIGH VERSUS LOW RELATIVE HUMIDITY

An experiment was designed to test the influence of relative humidity in the storage room upon the rate of development of *Phomopsis* and of *Diplodia*. Four samples of each of the varieties, Hamlin, Parson Brown, Pineapple, and Valencia, were obtained on March 17, 1944. One-half of the samples from each variety was placed in each of two storage rooms. The temperatures and relative humidities of the two rooms were controlled and obtained as shown in table 2.

The low humidity room was held at $75 \pm 0°$ F. and a relative humidity of 90 ± 4 per cent.

Isolations of *Phomopsis* and *Diplodia* were obtained from the rotten fruit at weekly intervals (Fig. 5). It was found that the least difference necessary for significance at the 5 per cent level of the 3-week and 5-week periods

TABLE 2.—*Temperature and relative humidities maintained in the 2 storage rooms*

Measurements	Low humidity room		High humidity room	
	Relative humidity (Per cent)	Temp., degrees F.	Relative humidity (Per cent)	Temp., degrees F.
Average of 5 weeks	56	75	90	79
Range of weekly ave.	±7	±0	±4	±3
Range of the Absolute Max. and Min.	±11–26	±3–2	+0–29	+10–18

was 10.7 per cent and 10.1 per cent, respectively, for *Diplodia;* and 11.9 per cent and 15.3 per cent for *Phomopsis.* The difference in amount of *Phomopsis* present in the two rooms was nonsignificant. The amount of *Diplodia* which developed in the rooms was different to an extent which was highly significant. The conclusion is that, for 5 weeks, the high humidity in the storage room caused an increase in the amount of *Diplodia* but not *Phomopsis.*

The development of *Phomopsis* and *Diplodia* was determined in fruit that was wet after it was picked and before it was stored. Samples of 50 fruits of the four varieties were soaked in water for periods varying from 5 to 30 minutes. At the end of 3 weeks in storage there were no significant changes in the numbers of fruits rotted by the two fungi as a result of the soaking. In 15 experiments an average of 10.07 fruits were rotted by *Phomopsis* among the soaked fruits and 7.0 among the fruits not soaked, while 7.0 fruits were rotted by *Diplodia* among the soaked fruits and 5.0 among those not soaked. The least differences necessary for significance were 3.26 for *Phomopsis* and 2.43 for *Diplodia.*

Gassing with ethylene for 48 hours had no significant effect on development of *Phomopsis* in stored fruit of the four varieties, but the *Diplodia* increased by an appreciable amount. In 50 fruit samples in 25 experiments the average number of fruits rotted by *Phomopsis* was 6.9 for gassed fruits and 5.5 for checks, and the least difference necessary for significance was 1.94. The average number rotted by *Diplodia* was 13.9 for the gassed fruits and 6.0 for the checks, the least difference necessary for significance being 3.31. An F value of 22.4 was highly significant.

EFFECT OF DIPHENYL WRAPS

In an experiment arranged to test the action of diphenyl wraps in controlling stem-end rot on oranges in various combinations of manner of picking and gassing, information was collected relative to the rate of development of both *Phomopsis* and *Diplodia.* The fruit was not subjected to a packinghouse process and was stored at 75° F. at about 75 per cent relative humidity. The various treatments and results obtained by analysis of the data at the 3-week period for variances are in figure 6. The treatments were arranged in the order of their rank of importance in allowing *Phomopsis* to develop. The least difference necessary for significance at the 5 per cent level is 7.5 per cent. It is evident that the wraps were not effective in controlling *Phomopsis.* In all cases there was a significant lowering of the incidence of *Phomopsis* in pulled fruit as compared with clipped fruit. A comparison of treatments 9, 10, and 6 to determine the difference in amount of *Phomopsis* caused by various methods of removing the calyxes shows that there is no difference. In this experiment gassing had no effect on *Phomopsis.*

The development of *Diplodia* in the same lots of fruit and the results obtained in these data are shown in figure 7 where 8 per cent is the least

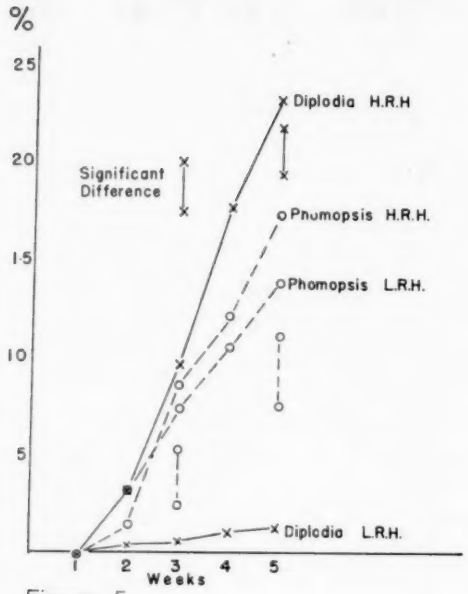

Figure 5.

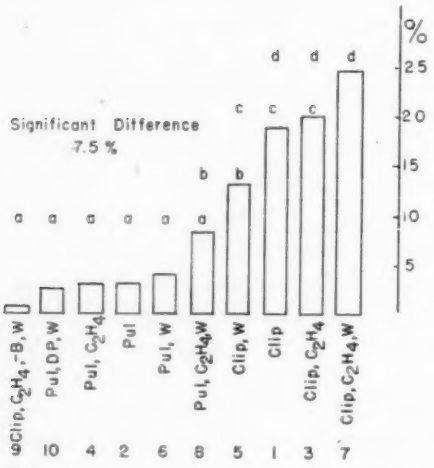

Figure 6.

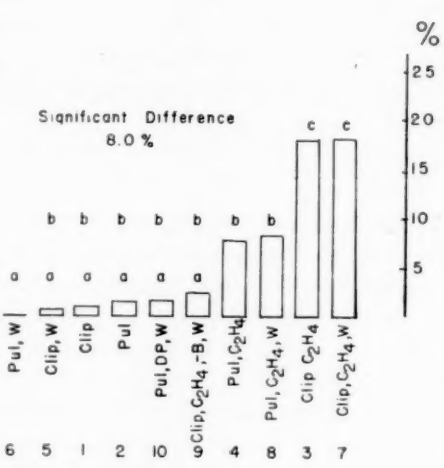

Figure 7.

FIG. 5. Rate of development of *Phomopsis* and of *Diplodia* on fruit in storage rooms having 90 per cent relative humidity (H.R.H.) or 56 per cent relative humidity (L.R.H.). Least difference necessary for significance for *Phomopsis* at 3 weeks, 11.9 per cent; at 5 weeks, 15.3 per cent; for *Diplodia* at 3 weeks, 10.7 per cent; at 5 weeks, 10.1 per cent.

FIGS. 6 AND 7. Development of *Phomopsis* (Fig. 6) and of *Diplodia* (Fig. 7) during 3 weeks in storage after various fruit treatments: Fruit picked by clipping (Clip) or by pulling (Pul); All buttons left on (+B) or calyx removed by reamer in drill press (DP); Fruit gassed with ethylene for 48 hrs. (C_2H_4); Individual diphenyl wraps on fruit (W).

difference necessary for significance. The wraps caused no difference in the amount of *Diplodia* present during 3 weeks in storage. When the fruit was not gassed there was no difference between pulled and clipped fruit. When the fruit was gassed, buttons left on, and fruit wrapped or unwrapped, there was less *Diplodia* in the pulled than in the clipped fruit. Gassing increased the amount of *Diplodia* in all cases except where the fruit was pulled and not wrapped.

SUMMARY

From approximately 4,000 isolations made from 850 separate samples of 50 fruits each, records were kept of the incidence of *Phomopsis citri* and of *Diplodia natalensis* as they occurred with various treatments. The fruit harvest was spread over the entire picking season and included the four main varieties of oranges, Hamlin, Parson Brown, Pineapple, and Valencia.

The samples were subjected to such treatments as: debuttoning, gassing with ethylene, soaking in borax, wrapping in diphenyl-impregnated paper, decalyxing by various methods, storage in high and low relative humidity, and various combinations of some of these treatments. The experiments were set up in such a manner that the data could be subjected to statistical analysis.

The 5 per cent level was chosen as the level of significance and with each experiment the least amount necessary for a significant difference between treatments was determined.

There was no correlation between the occurrence of *Phomopsis* and *Diplodia* in the same fruit.

The incidence of both *Phomopsis* and *Diplodia* was reduced by debuttoning, and by treatment with borax before, or before and after, gassing. Removal of only the calyx, not the entire button, reduced *Phomopsis* but not *Diplodia*. Gassing with ethylene did not control the fungi, and the incidence of *Diplodia* actually increased after gassing.

Borax alone reduced the incidence of *Diplodia* but not that of *Phomopsis*. Borax applied to gassed fruit, either before, or before and after gassing, also reduced the incidence of *Diplodia*.

Wetting the fruit after picking had no effect on *Phomopsis* or *Diplodia*. However, a high relative humidity during storage increased the amount of *Diplodia*.

Storing fruit in diphenyl treated wraps did not affect either of the fungi.

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BACTERIAL LEAF BLIGHT OF BIRD'S-NEST FERN

PETER A. ARK AND C. M. TOMPKINS

(Accepted for publication June 7, 1946)

Bird's-nest fern (*Asplenium nidus* L.) is grown on a large scale in commercial greenhouses in the San Francisco Bay region of California.

The writers' preliminary report on the bacterial disease of bird's-nest fern[1] is, apparently, the first record of a bacterial disease of ferns.

Bird's-nest ferns require two years to produce and are maintained in a constantly moist substratum in greenhouses in which high humidity and air temperature constantly prevail. There may be a rapid spread of the disease, often resulting in an epiphytotic. Losses to individual growers have in some seasons exceeded ten thousand dollars.

The disease starts as small, water-soaked, translucent spots on any part of the frond, but usually on the upper surface (Fig. 1). Infection may also occur through the water pores on the apical end of the frond. Under warm and humid conditions, the spots enlarge rapidly, soon involving the whole frond. Frequently, the bacteria invade the crown and quickly kill the plant. Necrosis of one or more fronds ruins the symmetrical appearance of the plant and thus renders it unsaleable.

The causal organism was readily isolated by macerating small bits of diseased tissue taken from the advancing margin of a lesion in sterile distilled water and plating out in potato-dextrose-peptone agar, on which the bacterium developed promptly and luxuriantly.

The pathogenicity of the isolated organism was proved in the following manner. Two isolates, one from an infection on the upper part of a leaf, and another from an infected crown, were grown on potato-dextrose-peptone agar slants at 28° C. After 24 hours, the growth was washed off with sterile distilled water (and poured) into a sterilized DeVilbiss atomizer. Small, healthy, bird's-nest ferns, grown in pots and tested for any chance infection by prolonged incubation in a moist-chamber, were inoculated. Before inoculation, the ferns were held in a large, glazed, moist-chamber with high humidity (90 per cent) for 24 hours, approximating that used by nurserymen. After spraying with the inoculum, the plants were returned to the tightly closed moist-chamber. The air temperature in the greenhouse fluctuated from 80° to 90° F. The first symptoms of the disease appeared as minute, translucent dots on the lower surface of fronds after three days. They increased rapidly, soon forming the large, translucent areas typical of the disease. When the ferns were kept in the moist-chamber 7 to 10 days fronds 3 inches in length were destroyed. When the ferns were transferred to a dry atmosphere the disease was promptly inhibited and there was little or no spread unless overhead watering was practiced. The check plants

[1] Ark, P. A., and C. M. Tompkins. Bacterial diseases of plants in California in 1945. U. S. Dept. Agr., Pl. Dis. Reptr. **30**: 28–29. 1946.

held under identical conditions and sprayed with sterile distilled water in a separate moist-chamber, remained healthy. Reisolations from sprayed plants yielded isolates which were identical with those used in the bacterio-

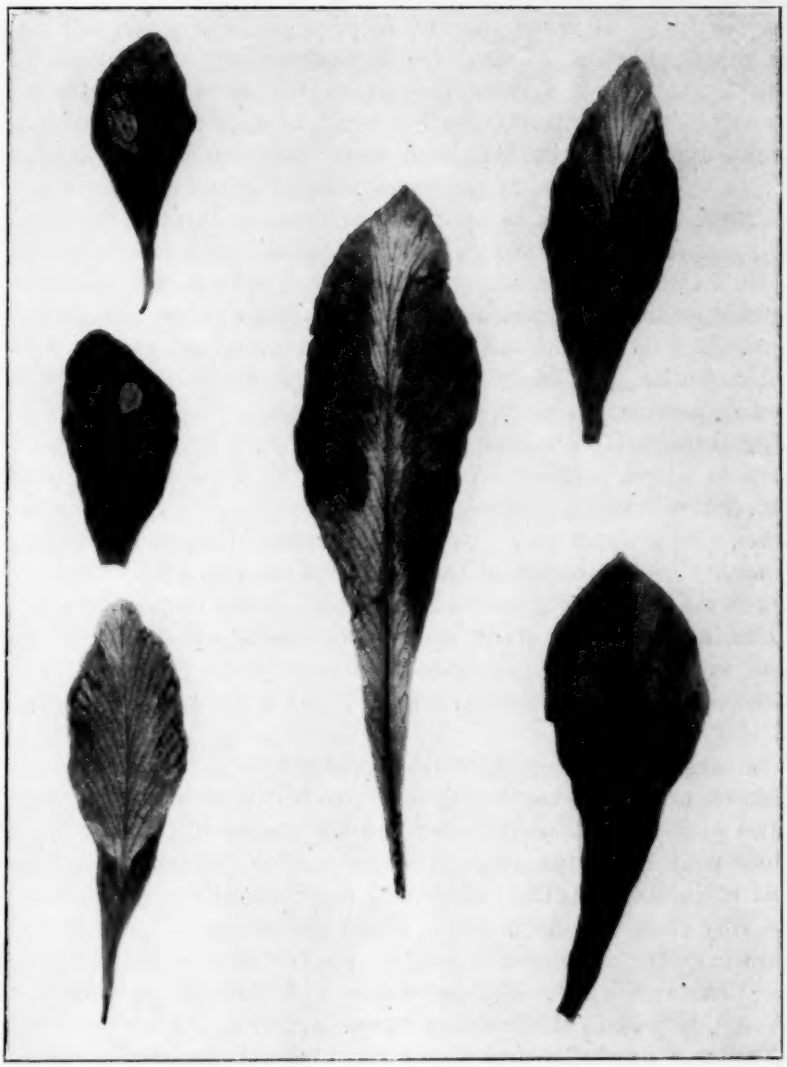

FIG. 1. Bacterial leaf blight of bird's-nest fern, *Asplenium nidus* L. Different stages in the development of the light, water-soaked lesions on the leaves. At lower right, bottom row, healthy leaf.

logical studies and produced, on inoculation, typical symptoms of the disease.

Six different isolates of proved pathogenicity and three reisolates were used in determining the bacteriological characters of the pathogen. Stand-

ard media and technique were used as described by the Society of American Bacteriologists.[2]

The bacterium is a short rod, 1.2–2.4 μ × 0.3–0.5 μ; Gram-negative; motile by means of 1 to 3 polar flagellae. On potato-dextrose-peptone agar plates, the organism grows rapidly, forming smooth, entire, flat colonies which rapidly increase in size. On beef-extract-peptone agar slants, the growth is grayish-white, with fluorescence in the medium. On potato-dextrose-peptone agar slants, growth is rapid, heavy, strongly grayish-white, butyrous; the medium darkens with time. Nutrient broth (beef-extract-peptone) is turbid within 24 hours; no pellicle; crystals are observed with age. There is no curdling of skimmed or litmus milk. In litmus milk, a white precipitate is observed on long incubation. Indol is not formed in Dunham's solution. Ammonia is formed in peptone-glucose-dipotassium phosphate media as suggested by Hansen.[3] Nitrates are not reduced to nitrites. H_2S is not formed in Bacto lead acetate agar. In tubes of Fermi's solution, previously sterilized by autoclaving for 15 minutes at 15 pounds pressure, to which the following sugars, previously sterilized by filtering through UF sindered filters, and inverted Dunham fermentation tubes were added, acid but no gas is formed from the following carbohydrates: dextrose, sucrose, maltose, arabinose, xylose, glycerin, galactose, and fructose. Very slight acidity develops in lactose broth on long incubation. Very scanty growth occurs in raffinose broth, and no acid. Good growth occurs in the following synthetic media: Soc. Amer. Bact., Fermi's, Cohn, and Uschinsky. Starch is not hydrolyzed. Gelatin is liquefied. The organism is a facultative anaerobe. Thermal death point, 50° C. The organism develops at temperatures from 1° to 34° C., with optimum growth at 22°–30° C.

The name *Phytomonas* **asplenii** n. sp. is proposed.

Control of this disease is entirely dependent upon sanitation and good cultural conditions. The pathogen can be introduced into the brick-dust medium used for propagation purposes on fern spores taken from old fronds of infected plants. Therefore, in propagating, spores should be taken only from healthy plants. Where the disease is present it is important to destroy all plants with symptoms and to grow the healthy plants under drier conditions. The brick-dust and ratsnest (a natural forest compost), flats, and pots should be steam-sterilized.

When the prothallia and young plants are being transplanted, they should be handled with forceps which are frequently flamed in alcohol.

Tests in several commercial greenhouses in San Francisco over a period of two years proved conclusively that sanitation and improved cultural conditions will yield healthy bird's-nest ferns.

[2] Society of American Bacteriologists. Manual of methods for pure culture study of bacteria. Leaflet II. Preparation of media. 9th ed. 1944.

[3] Hansen, P. A. The detection of ammonia production by bacteria in agar slants. Jour. Bact. 19: 223–229. 1930.

SUMMARY

1. A bacterial disease of bird's-nest fern causes serious losses in greenhouses in central California.

2. A bacterial organism isolated from the advancing margins of the lesions proved pathogenic upon inoculation and was readily reisolated.

3. The causal organism is described and the name *Phytomonas asplenii* n. sp. is proposed. The colonies are grayish-white. The organism is motile by flagellae attached to one pole.

4. Control measures consist of strict sanitation involving steam-sterilization of the brick-dust and ratsnest media, flats, and pots, the use of sterile forceps in transplanting, and avoidance of excessive watering and too high humidity in the greenhouses.

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WITCHES' BROOM OF ALFALFA IN NORTH AMERICA[1]

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(Accepted for publication June 10, 1946)

INTRODUCTION

The witches' broom disease of alfalfa (*Medicago sativa* L.) has been recognized in the United States since 1925. A disease of the same name and symptomology also occurs in Australia where it has been studied in detail by Edwards (5, 6). The disease in America has received very little attention because of its sporadic occurrence and minor importance. Recently, however, it has become a serious problem in localized areas of Washington (15) and of British Columbia (2) in Canada. Edwards showed that the Australian witches' broom is a virus disease, transmissible by grafting but not by mechanical means. The present studies were undertaken to clarify the relationship between the American and Australian alfalfa witches' brooms and to further investigate methods of transmission.

HISTORY AND DISTRIBUTION

Most of the previous references to alfalfa witches' broom in America are records of occurrence only, with occasional descriptions of symptoms. Although most reporters considered the symptoms to be indicative of a virus disease, it was not until Heald and Wellman (10) reported graft transmission in 1939 that this belief received experimental support.

The first North American record of this disease appears to have been a report by Haskell (8) of a witches' broom on alfalfa in Idaho in 1925. According to Richards (16) the disease appeared in Utah about the same time. In 1932, in a brief report, Foster (7) mentioned the occurrence of witches' broom in two widely separated areas in British Columbia. Other reports show that the disease was first found in Washington in 1934 (9) and in Alberta in 1940 (4). It has been found occasionally in the Umatilla Valley of Oregon but the history of witches' broom in that State is not known. Recently this disease has been reported from Arizona by Hoyman (11) who, in 1943, found less than 1 per cent in one of the older alfalfa plots located at the University of Arizona Farm on the Yuma Mesa. Dr. Hoyman states, in correspondence, that witches' broom was not observed in younger stands on the Mesa or elsewhere in the State.

These reports limit the known distribution of witches' broom to the area between the Cascade and Rocky Mountains except for an eastern extension

[1] Published with the approval of the Director of the Washington Agricultural Experiment Stations as Scientific Paper No. 659. Includes portions of a thesis submitted to the graduate school of the State College of Washington in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2] Formerly Assistant Plant Pathologist, Washington Agricultural Experiment Stations, now Pathologist, Division of Soils, Fertilizers, and Irrigation, Bureau of Plant Industry, Soils, and Agricultural Engineering. Grateful acknowledgment is made to Dr. F. D. Heald for helpful advice and encouragement and to Mr. H. P. Singleton for cooperation in the field studies and for taking several of the photographs.

of the disease into Alberta. A single specimen of a witches' broom on alfalfa has been reported from Vancouver Island (7). Within this general area witches' broom has some very interesting features of distribution and occurrence. Although the disease is widely scattered in the Northwest, it has reached serious proportions in only 3 or 4 relatively isolated areas. The development of the disease in Utah, Idaho, Washington, and British Columbia has a rather consistent pattern.

The Utah outbreak was the first to occur. According to Richards (16) the disease was first seen in Salt Lake County in 1925. By 1928 it was considered an important factor in reduction of stands. Dr. Richards reports, in recent correspondence, that although the disease at one time could be found in older fields to the extent of 60 to 65 per cent of the plants, it has now almost entirely disappeared, being found only along ditch banks and fences. This reduction is correlated with short rotations adopted for bacterial wilt control but it seems unlikely that so striking a control of witches' broom could be obtained in this manner.

Dr. E. C. Blodgett has recently furnished the author with a summary of the witches' broom situation in Idaho. Following the original record of the disease in Elmore County in 1925 no further observation of witches' broom was made until Blodgett noted it along the Snake River in the same county during a plant disease survey in 1944. Although the survey included most alfalfa areas of Idaho, the disease was not found elsewhere in the State. Blodgett found many fields in the Elmore County area wherein infections ranged from a trace to 50 per cent. The disease is important in the area because of the resulting reduction of stands.

In Washington, scattered infections have been noted in Walla Walla, Benton, Franklin, Yakima, Grant, and Okanogan counties, but except for moderate severity in small plots at Prosser (Benton County) the disease is important only in the Methow Valley area of Okanogan County, where it was first reported in 1934 (9). In 1939 Heald and Wellman (10) reported infections in this area ranging from 25 to 60 per cent. Later surveys by the author show that the disease continued to increase during the next 3 years. Infections during those years reached as high as 80 per cent with accompanying stand reductions. Some growers reported having to plow and reseed fields in as short a time as 3 years because of witches' broom infection.

The Methow Valley is narrow and isolated from the more open alfalfa areas in central Washington. The original outbreak of witches' broom was at the upper end of the valley near Mazama. Since then it has spread to involve almost all the alfalfa areas of the valley to a point about 30 miles below Mazama. Since 1942 there is evidence that new plantings in the center of the original diseased area are being less severely attacked, although infections in more recently invaded areas are still at a high level. Young stands in the Mazama area now have only scattered infections whereas in previous years growers despaired of maintaining stands in these fields be-

cause of the severity of the disease. The topography of the area is very irregular and it is, therefore, difficult to decide whether or not there is a "crest" of infection moving outward from the original point of occurrence. The rate of spread down the valley during the last 3 years has been slow, and has now reached a natural barrier of rough, uncultivated territory which may effectively isolate the disease from the other alfalfa areas of the Columbia Valley.

The information available on the distribution of witches' broom in British Columbia (2) shows that infections have built up in the Nicola Valley comparable to the Methow Valley area. Here, again, severe disease development has been strikingly localized.

Witches' broom, therefore, has a 20-year history of persistent localization of severe outbreaks. The natural recession of the disease in Utah and the indications of the same phenomenon in Washington are hopeful signs that the same may occur in other areas. The factors responsible for witches'-broom outbreaks must be common to these areas but not general within the known distribution range. These factors are not known, but it seems most likely that they are concerned with the population trends of insect vectors.

A witches' broom on sweet clover has been reported in Alberta (3) but has not been found in Washington even in areas where alfalfa is seriously affected. Very similar symptoms, however, occur on red clover and White Dutch clover in Washington and have been seen occasionally also on black medic (*Medicago lupulina* L.). No hosts other than alfalfa have been reported for the disease in other states or in Australia.

SYMPTOMS AND EFFECTS

Witches' broom of alfalfa is a disease which slowly modifies the appearance of affected plants during the course of one to several seasons. Consequently a wide range of symptoms is encountered in the field. In the typical or advanced stage of infection the plants are severely dwarfed and bunchy (Fig. 1, B). This is due to the excessive development of short spindly shoots from all parts of the crown and from axillary buds along the stems. The plants have a yellowish cast as a result of marginal chlorosis of the leaves and a general pale-green color of the tiny succulent stems. Normal alfalfa leaflets may be described as oblong or elliptic-oblanceolate with spinose denticulations toward the apex. Leaflets on affected plants are definitely smaller, obovate to cuneate, usually lack apical denticulations, and are frequently wrinkled or puckered as shown in figure 2, A. Usually, witches'-broom-infected plants are erect and compact but may develop a prostrate habit of growth as the tops approach maturity. Diseased plants are easily recognized in the field, even from a considerable distance, especially if the stand is thin or newly cut.

The first symptoms of infection usually are seen after new growth commences following cutting. Plants producing symptoms for the first time

invariably have the same degree of infection over the entire plant. There may be little or no dwarfing but the plants can be recognized immediately by

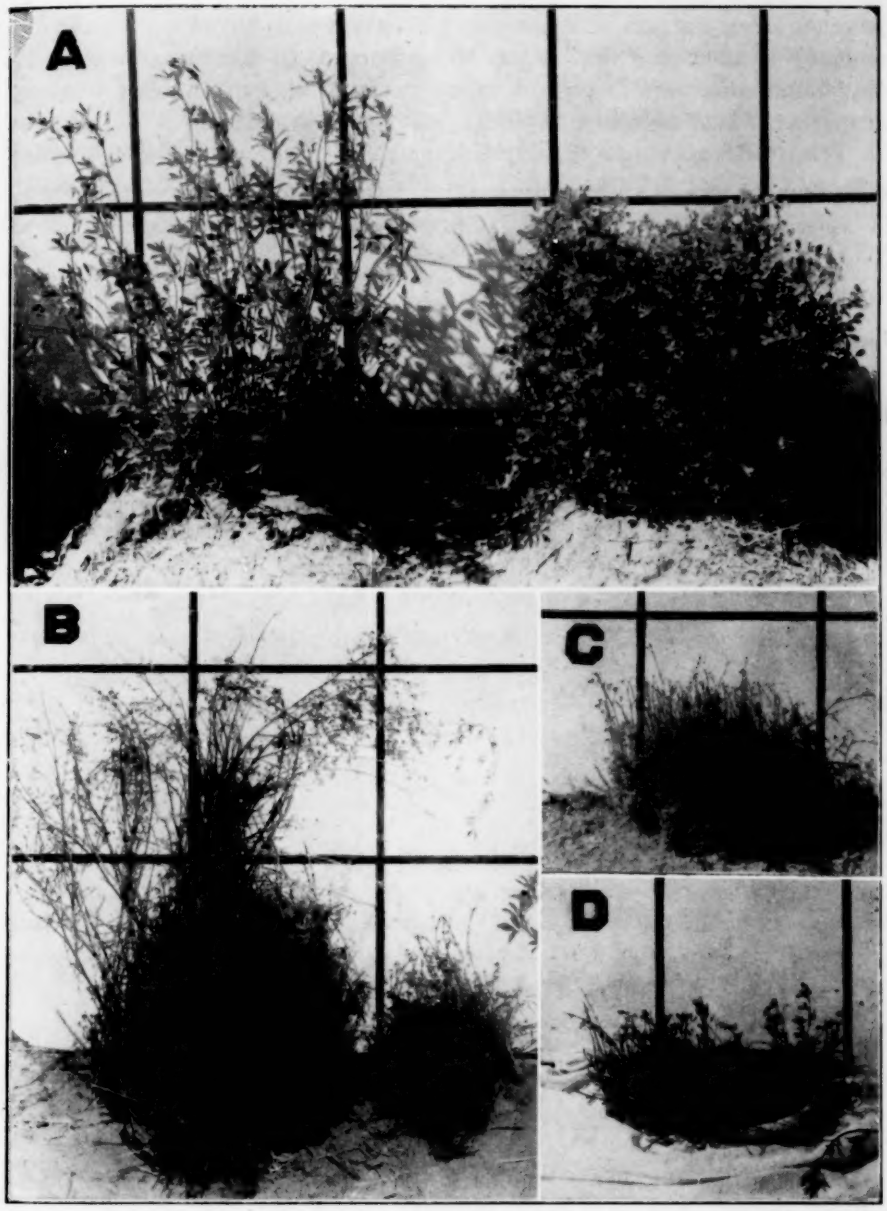

FIG. 1. Alfalfa witches' broom. A. Early stage of infection (right) contrasted to healthy plant (left). B. Two severely diseased plants, the larger one with tufts of witches' brooms on previously healthy stems. C. Advanced stage of witches' broom. D. Severe symptoms of bacterial wilt for comparison. All photographs by H. P. Singleton.

the greatly increased number of stems and erect habit as well as by a slight marginal chlorosis of the younger leaves. After the next cutting the new

growth will usually be decidedly dwarfed and the leaflets much reduced in size. Diseased plants become progressively stunted after each cutting and the number of stems is increased. Several hundred spindly stems are common on infected plants while severely infected crowns have been found with as many as 3,000 very fine, densely matted stems. Such plants can scarcely be recognized as alfalfa because of severe dwarfing and an accompanying reduction of leaflets to tiny, bract-like structures on the ends of the petioles.

While affected shoots usually originate from the crown they may also

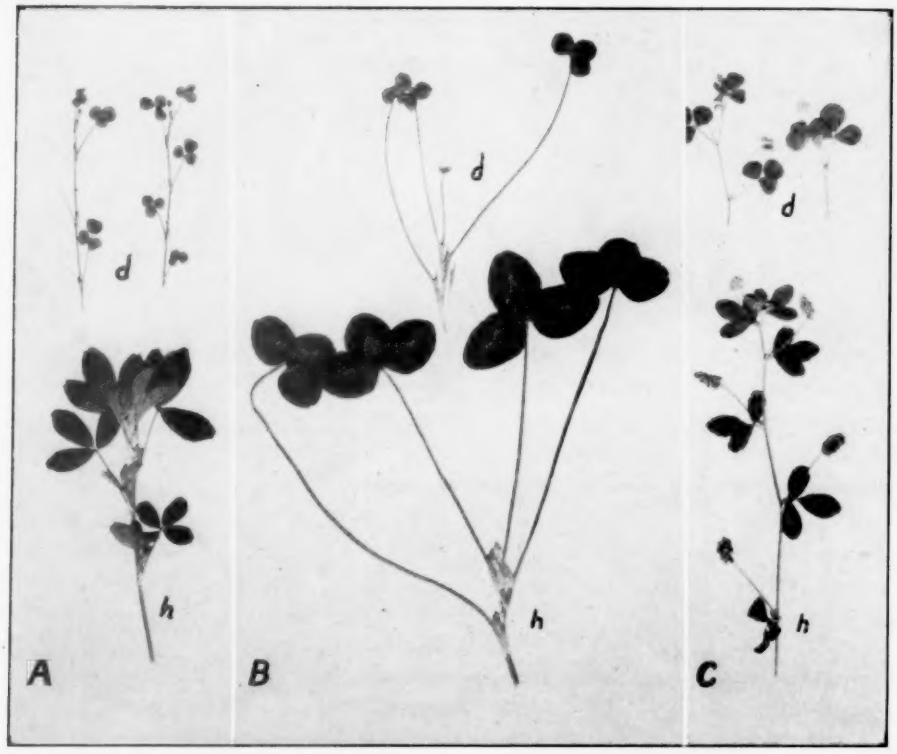

FIG. 2. Symptoms of alfalfa witches' broom obtained by graft transmission. A, on alfalfa; B, on red clover; C, on black medic (*M. lupulina*). Healthy shoot (h), diseased shoot (d).

appear in tufts at the nodes on previously healthy stems. This condition occurs on newly infected plants when the old, symptomless growth is not removed by harvesting. A general view of such a plant is shown in figure 1, B.

Witches' broom causes a definite decrease in yield because of the dwarfing but the chief source of loss is the reduction of stands by the early death of diseased plants. Plants die during the winter, which suggests that the infected plants are so weakened by the constant proliferation of new shoots that they readily succumb to low temperatures. Data were obtained on death rate of diseased plants in a row-planted nursery at the Irrigation

Branch Experiment Station, Prosser, where it was possible to follow the course of the disease in individual plants.

During a 3-year period 131 newly infected plants were observed during one winter, 54 were observed through 2, and 17 through 3. In the first group 50 per cent of the plants died during the first winter. In the second group 39 per cent died the first winter and 81 per cent were dead after the second. In the third group the rate of death was 35, 59, and 90 per cent at the end of 1, 2, and 3 winters, respectively. Thus, in these cases, almost half the infected plants died the first winter and only 10 per cent survived three winters. The decrease in number of healthy plants in the same nursery during the same 3-year period was from 2,507 to 2,078, an average annual death rate of approximately 6 per cent.

Seed production is almost entirely inhibited in diseased plants. The few flowers which are produced are usually in groups of two or three instead of in many-flowered racemes typical of normal plants. The blossoms are smaller and paler than the normal and rarely produce seed. The small quantity of seed collected from infected plants has been poor in appearance but has germinated well.

TRANSMISSION EXPERIMENTS

Mechanical Transmission Tests

Neither Edwards (6) nor Heald and Wellman (10) were able to transmit witches' broom by mechanical inoculation methods. Edwards used various techniques involving the use of cotton swabs, pin pricks, and hypodermic needle injections into stems and roots. The method used by Wellman was probably the one recommended by Jones (13) as being sometimes effective when other methods fail. This consists of using macerated diseased tissue on a swab as inoculum and dusting carborundum powder on the leaves to be inoculated.

This method was used by the author to inoculate 250 plants but no infection resulted. During the five years of this study many healthy and diseased plants have been handled in the greenhouse without regard to the possibility of accidental mechanical transmission, yet none is known to have occurred. In unsuccessful grafting attempts where diseased scions have remained alive for as long as 10 days before withering, there has been no transmission of the disease. It therefore seems unlikely that mechanical transmission can occur and certainly there is little possibility of the disease being spread in the field by the mower or other mechanical means.

Graft Transmission Tests

Both Edwards and Heald and Wellman were successful in transmitting witches' broom by grafting. However, since it could not be assumed that the Australian disease was the same as the one in the United States, and since the work of Heald and Wellman was incomplete, grafting tests were repeated on a more extensive scale. Edwards used a wedge- or veneer- root-

grafting technique yielding about 12 per cent successful grafts. Heald and Wellman also used various types of root grafting with methods found to be satisfactory by Weimer (17) in grafting studies on alfalfa dwarf.

Preliminary root-grafting tests were tried but because of a low percentage of successful grafts, the method was abandoned in favor of a modification of the shoot-grafting technique. Because of the slenderness and succulence of witches'-broom-infected shoots the usual cleft-graft method as used on more robust herbaceous plants was not satisfactory. The chief change necessary in using the method was to keep the delicate grafts in a humid atmosphere in a glassed humidity chamber for at least 10 days after grafting. The unions were carefully but firmly bound with fine thread which could later be removed. After considerable practice it was possible to obtain as high as 75 per cent successful grafts by this method.

TABLE 1.—*Time required for sufficient tissue union in alfalfa grafts to allow transfer of the witches'-broom virus*

Duration of graft	Transmission results	
	Total plants	Plants infected
days	*number*	*number*
5	5	0
10	5	0
12	5	2
16	5	3
20	5	4
25	4	4

In all cases where diseased scions became established on healthy alfalfa plants, transmission of witches' broom was obtained. In one typical experiment with 5 root-approach grafts the disease was transmitted in 118 days while with 23 shoot grafts it was transmitted in an average of 61 days. The results with the root grafts are in accord with those of Edwards who reported a latent period of the virus in the plant of from 4 to 5 months. The delayed transmission with the root grafts may be due in part to a greater length of time required for adequate tissue union.

The actual latent period between inoculation and symptom expression is difficult to determine in the case of alfalfa witches' broom because of indefinite early symptoms. Thus, the latent period ranged from 32 days to 137 days in the case of shoot grafts, a variation which appeared to be related to growth rate of the plants, faster growing plants producing the earliest symptoms. Since these figures also include the time necessary for sufficient tissue union to allow virus transfer, a scion-removal experiment was performed to determine this interval. Thirty young alfalfa plants were grafted by the shoot-graft method with scions affected with the disease. Five days after grafting the scions were removed from 5 of the plants and from groups of 5 others at intervals of 10, 12, 16, 20, and 25 days. All plants were kept in the greenhouse for 4 months before final records were taken.

Table 1 shows that virus transfer commenced between the 10th and 12th day and had taken place in all plants in from 20 to 25 days. Thus, under the conditions of these tests the actual latent period of the virus in the plants averaged approximately 40 to 50 days.

Studies of Host Range by Means of Grafting

The report of witches'-broom symptoms on sweet clover in Alberta and the occurrence of similar symptoms on red clover, White Dutch clover, and black medic in Washington led to attempts to determine the leguminous host range of the virus by means of cross-grafting. This method has been used successfully with solanaceous plants but has not been very successful in other families beyond generic limits.

In the fall of 1941, some preliminary grafts were made on 2 species of sweet clover (*Melilotus alba* and *M. officinalis*) using witches'-broom-infected alfalfa scions. Eventually 12 scions were established on plants of *M. alba* and 7 on *M. officinalis*. These plants were observed in the greenhouse for one year. During this time the growth of scions on *M. alba* was stunted, but the unions appeared to be sound. No symptoms of witches' broom appeared on the sweet clover and back grafts to alfalfa indicated the absence of virus. The stocks of *M. officinalis* died during the year without any evidence of virus transmission.

During the winter of 1942–43 numerous cross grafts from diseased alfalfa to various species of legumes were attempted. Although many of these grafts failed to survive longer than the initial humidity treatment, eventually 87 apparently successful grafts were obtained on 12 different species of legumes. At the same time a few additional grafts were made on alfalfa as a check on the technique.

Although the numerous alfalfa scions on different legume species seemed to be well established, it eventually became evident that all alfalfa scions on genera other than *Medicago* had failed to make union but were being maintained on their own adventitious roots which had become anchored in the pith tissue of the stock. The final results both in scion rooting and transmission are summarized in table 2. The fact that the scions were producing roots rather than uniting with the stock became evident when living alfalfa scions were noted on red clover stems that had died. On red clover the scion roots were very fine and could be mistaken for vascular strands of the clover, in other cases the scions produced such vigorous roots that the stock stem was eventually split and shredded and the roots became anchored in the soil. In the case of the one surviving scion grafted on bean there was no external evidence of rooting even 3 months after grafting and although the scion was not thrifty it was still living. At the end of this period the bean stem was cut open revealing a single, stout alfalfa root about three inches long extending through the pith tissue of the stem but not reaching the soil. Although rooting of scions was not common on *Medicago* species in this test, later grafts with alfalfa resulted in several

cases. Microscopic examination of many grafts of diseased scions on healthy alfalfa revealed a frequent tendency for root primordia to form, but root development appeared to be checked by satisfactory tissue union. In one case scion rooting was accompanied by sufficient tissue union for virus transmission. Since healthy alfalfa scions were not used in any of these grafts it is not known what effect the virus may have in stimulating the rooting tendency. However, back grafts with apparently healthy scions of red clover and white sweet clover onto healthy alfalfa also gave the rooting response without tissue union.

These results show that scion survival in cross grafts with the legumes used does not constitute sufficient evidence for tissue union. Consequently use of the method for studying virus host range in this family does not appear promising except within genera.

TABLE 2.—*Results of cross-grafting experiments with witches' broom infected alfalfa scions on various species of legumes*

Stock species	Plants with living scions			Good union	Scions rooted	Virus transmitted
	After 12 days	After 30 days	After 90 days			
	no.	*no.*	*no.*	*no.*	*no.*	*no.*
Medicago sativa L.	13	10	8	6	2	8
M. lupulina L.	10	5	5	5	0	5
M. hispida Gaert.	4	4	3	3	0	3
M. arabica Huds.	8	4	3	3	0	?[a]
Trifolium pratense L.	12	12	8	0	8	0
T. fragiferum L.	4	2	0	.....	.....	0
T. hybridum L.	10	8	3	0	3	0
T. repens L.	7	5	0	.....	.....	0
T. incarnatum L.	10	10	5	0	5	0
Lotus corniculatus L.	6	0	0	.....	.....	0
Onobrychus sp.	5	4	1	0	1	0
Phaseolus vulgaris L.	4	2	1	0	1	0
Robinia Pseudo-Acacia L.	7	1	0	.....	.....	0

[a] Plants matured and died without showing disease symptoms.

The cross grafts to other species of *Medicago* were successful as evidenced by good scion growth and absence of rooting. Five grafts out of 10 attempts were obtained on *M. lupulina* with transmission of the virus in each case.

The symptoms, as shown in figure 2, C, are similar to those produced on alfalfa. Back grafts from these plants to healthy alfalfa produced the typical symptoms of the disease in the latter. With *Medicago hispida* 3 successful grafts were obtained followed by the appearance of witches'-broom symptoms in the stock after an interval of approximately 3 months. Although 3 successful grafts were obtained on *M. arabica* no appearance of symptoms was recorded prior to the death of the plants. Since this species is an annual it is possible that the grafting was done too late for symptoms to develop before the plants matured. Previous mention has been made of the natural occurrence of witches' broom on red clover and White Dutch clover.

The inability to obtain grafts between diseased alfalfa and either red clover or White Dutch clover prevented experimental proof of the relationship between the naturally occurring witches' brooms on these 3 hosts. Infected plants of these 2 clovers were used in the greenhouse for various grafting tests. No permanent grafts with witches'-broom scions of white clover were obtained on any species. Diseased red clover scions, however, were grafted to healthy red clover with transmission of the typical witches'-broom symptoms as shown in figure 2, B. Since the clover witches' brooms are found only in association with alfalfa witches' broom and since the red clover form is also graft-transmissible, it is very likely that they are all caused by the same virus.

The host range of alfalfa witches' broom, therefore, definitely includes *Medicago lupulina* and *M. hispida*, probably includes *Trifolium repens*, *T. pratense*, and according to Cormack (4), also includes *Melilotus sp.*

Insect Transmission Tests

The only previous attempt to find an insect vector for alfalfa witches' broom was by Edwards (6). In most of his experiments mass collections of insects from alfalfa fields were allowed to feed at will on both healthy and diseased plants under large cages. No transmission was obtained with any of the insects used. Since field observations suggested the presence of an active insect vector, tests were begun in Washington in 1941 with sucking insects common in alfalfa fields. A report has already been made on preliminary evidence that the leafhopper, *Platymoideus acutus* Say, can transmit this disease (14). Since the details of the tests were not published, they are reported here.

In the exploratory vector tests, mass collections of suspected species were caged on infected alfalfa plants for a short feeding period after which they were segregated into species groups and transferred to healthy plants. No attempt was made to obtain identification of insects giving negative results since such tests were not considered conclusive.

In the course of several hundred tests with sucking insects one case of transmission occurred where *Platymoideus acutus*[3] was used. In this case a large, but undetermined, number of leafhoppers of this species was caged on an infected plant from May 26 to June 2, 1943, after which they were transferred to a healthy plant for 12 days. At the end of this time 7 insects were still living. Witches'-broom symptoms were first considered definite on August 14, approximately 2 months after inoculation.

A second experiment was then begun using larger cages, in which one infected plant and 4 to 6 healthy plants were placed. Field collections of the suspected species were added to some of the test cages from time to time during August and early September, while other cages were held as checks. By the end of October all the insects had died and the plants were removed to the greenhouse bench for observation.

[3] Identification of this insect was kindly made by Dr. R. H. Beamer, University of Kansas.

No symptoms appeared on these plants until early in February, 1944, when 2 plants were recorded as suspicious. Symptoms continued to develop on these and other plants until finally, by May 15, eighteen of the 29 surviving test plants were definitely affected with witches' broom. None of the 6 check plants became diseased and no infections appeared on uncaged healthy alfalfa that had been held in the same greenhouse during the entire period of the test.

A third series of tests with the same leafhopper was carried out during the summer of 1944. Small cages to fit 6-inch pots were used. Each pot contained from 5 to 10 alfalfa seedlings grown from seed under the cages. Details of each test and the transmission results are shown in table 3.

TABLE 3.—*Results of witches'-broom transmission tests on alfalfa seedlings with the leafhopper Platymoideus acutus Say. during the summer of 1944*

Pot No.	No. of alfalfa seedlings	No. of insects		Source of insects	Feeding period	No. of plants infected
		Adults	Nymphs			
1	5	10	1	Diseased plt.[a]	21 days	4
2	9	4	1	do	indef.	4
3	5	0	3	do	21 days	3
4	6	1	20	Field coll.[b]	indef.	3
5	6	5	25	do	indef.	0
6	6	1	15	do	17 days	4
7	5	0	3	do	17 days	0
8	6	1	2	do	18 days	0
9	4	0	3	do	18 days	0
10	5	Check—no insects		.....	.....	0
11	4	do		.....	.....	0
12	5	do		.....	.....	0
13	5	do		.....	.....	0
14	6	do		.....	.....	0
15	8	do		.....	.....	0

[a] Insects caged on an infected plant for at least 10 days.
[b] Insects taken from an alfalfa nursery with approximately 50 per cent witches' broom.

The percentage of transmission obtained in the various cages of the third test appears to bear some relation to the number of insects. The mortality rate was high in all cages and may account for the poor transmission recorded in cages where only a few insects were used.

The consistent transmission of witches' broom in all tests with *Platymoideus acutus* and the absence of transmission in the check cages establishes this insect as a vector of the virus. Certain field observations, however, suggest that it may not be the most important natural vector. The insect could not be found in the Methow Valley during 2 visits there in the summer of 1944. These searches were made in June and August, when the insect could be found in the Yakima Valley, and included sweepings in fields very severely infected with witches' broom. Furthermore, field records at Prosser indicate a peak of new infections early in the season whereas the peak of population of *P. acutus* occurred in late August. Even though this species may not be the chief vector its widespread occurrence provides a

possible means by which witches' broom could become established in new areas.

Dodder Transmission Tests

The ability of various species of dodder (*Cuscuta spp.*) to act as transmission bridges for certain viruses has been demonstrated by Bennett (1), Johnson (12), and others. Since dodder was found generally distributed in alfalfa fields in the Methow Valley, the possibility that dodder might transmit the witches'-broom virus was investigated.

Field-grown *Cuscuta campestris* Yunk. was established in the greenhouse on 9 rooted cuttings of witches'-broom-infected alfalfa in small pots. After the parasite had made sufficient growth the dodder strands from each plant were trained across to a healthy alfalfa plant in an adjacent pot. Nine such pairs were established each having several well connected dodder bridges. The period of union varied from 17 to 62 days after which the dodder was removed and the healthy plants kept for observation. None of these plants subsequently developed symptoms of witches' broom, which indicates that this species of dodder is not a factor in transmission of the virus.

Seed Transmission Tests

To test the possibility of seed transmission of the witches'-broom virus, collections of seed were made from infected plants. These plants rarely set seed and it was possible to collect only a very small amount for this purpose. In 2 different tests, 488 plants were grown from this seed and kept for at least one year in a nursery. None of the plants had any evidence of witches'-broom infection.

COMPARISON OF AMERICAN AND AUSTRALIAN WITCHES' BROOM

No positive evidence has been obtained to show that the witches' broom described by Edwards in Australia differs from the one in the United States. The symptoms appear to be identical and the transmission experiments show similar agreement where positive data are available for both diseases. Edwards reported a phylloid condition of blossoms on infected plants which he considered to be part of the symptom picture. A similar phyllody has been observed in Washington on both witches' broom and healthy plants and is therefore believed to be unrelated. Edwards also reports occasional recovery of diseased plants when transplanted to the greenhouse, a phenomenon that has not been observed here. Those possible differences require further study before they can be evaluated. In the meantime it is justifiable to assume that the 2 diseases are the same.

SUMMARY

Alfalfa witches' broom is a virus disease occurring in Western United States and adjacent Canada. It appears to be the same as the alfalfa witches' broom prevalent in southern Australia. Comparison of known features of the two forms of disease shows no positive differences.

This disease has become serious in isolated areas of the Pacific Northwest but has not spread widely within this area since its first appearance 20 years ago. The history of the disease indicates sporadic outbreaks followed by natural recession.

Affected plants are decidedly dwarfed and have a dense proliferation of shoots from the crown. Death follows in from one to three years. With heavy infection, stands are rapidly reduced.

Witches' broom occurs naturally on alfalfa, red clover, and White Dutch clover. It has also been reported on sweet clover in Alberta. Grafting tests have resulted in artificial infection of *Medicago lupulina* and *M. hispida*.

The virus has been transmitted by both root and shoot grafting and by means of the leafhopper *Platymoideus acutus* Say. Negative results were obtained in efforts to transmit the witches'-broom virus by mechanical means, by dodder, or through the seed.

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PHYTOPATHOLOGICAL NOTES

An Undescribed Coryneum on Diseased Italian Cypress.—An undescribed species of *Coryneum* was found on an Italian cypress (*Cupressus sempervirens* L.) from the vicinity of Montgomery, Alabama. Although dead terminal buds, leaves, and branchlets of the cypress gave evidence of a diseased condition, the pathogenicity of the fungus has not been investigated. The fruiting bodies of the fungus appear as small black pustules scattered sparingly on the leaf surfaces of the host or occasionally coalescing on the leaf margins. No perfect stage was observed.

Coryneum **asperulum** sp. nov.[1]

Acervuli conspersi, atri, erumpentes, pulvinati, plerumque circulares, 50–200 μ in diam.; conidia oblongo-fusoidea, asperula, typice 3-septata, olivaceo-grisea, 27–32 μ × 5–6 μ (19.0–40.6 μ × 3.6–6 μ), ad septa non- vel subconstricta, cellula terminali ad apicem muticum leniter attenuata, cellula basali truncata; conidiophora simplicia, brevia; pseudoparaphyses nulli.

In foliis morbosis Cupressi sempervirentis, Alabama.

Acervuli scattered, black, erumpent, pulvinate, usually circular, 50–200 μ diameter; conidia oblong-fusoid, slightly roughened, typically 3-septate, deep olive-gray to dark olive-gray, 27–32 μ × 5–6 μ (19.0–40.6 μ × 3.6–6 μ), not or only slightly constricted at the septa, terminal cell slightly tapering to a muticate apex, basal cell truncate; conidiophores simple, short; pseudoparaphyses absent.

On diseased foliage of *Cupressus sempervirens* L.: Alabama. Type (F. P. No. 48276) in the Mycological Collections of the Bureau of Plant Industry, Soils, and Agricultural Engineering, United States Department of Agriculture, the New York Botanical Garden, and the Farlow Herbarium of Harvard University. The general structure of the fruiting body is shown in figure 1, A and B, with spores shown in C.

Twelve species of *Coryneum* have been described on conifers; of these, two occur on *Cupressus*. Wagener[2] reported *Coryneum cardinale* Wagener as the cause of serious stem and branch cankers on the Monterey cypress (*Cupressus macrocarpa* Hartw.) and, occasionally, on the columnar Italian cypress (*C. sempervirens* L. var. *stricta* Ait.) in California. Natural infections were observed on *C. pygmaea* (Lemmon) Sarg., *C. forbesii* Jeps., and *C. lusitanica* Mill.; and artificial inoculations extended the list of susceptible species and varieties of *Cupressus*. *Coryneum berckmanii* Milbrath, which causes a blight on the foliage and young stems of ornamental varieties of *Thuja orientalis* L., occasionally affects the columnar Italian cypress in Oregon.[3] Both *C. cardinale* and *C. berckmanii* differ from *C. asperulum*

[1] The Latin description was prepared by Edith K. Cash, Associate Mycologist, Division of Mycology and Disease Survey, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture.

[2] Wagener, Willis W. The canker of *Cupressus* induced by *Coryneum cardinale* n. sp. Jour. Agr. Res. **58**: 1–46. 1939.

[3] Milbrath, J. A. Coryneum blight of Oriental Arborvitae caused by *Coryneum berckmanii*, n. sp. Phytopath. **30**: 592–602. 1940.

by having 5-septate spores. Only three of the twelve species reported on conifers belong to the 3-septate group. These are *C. bicorne* Rostr. reported

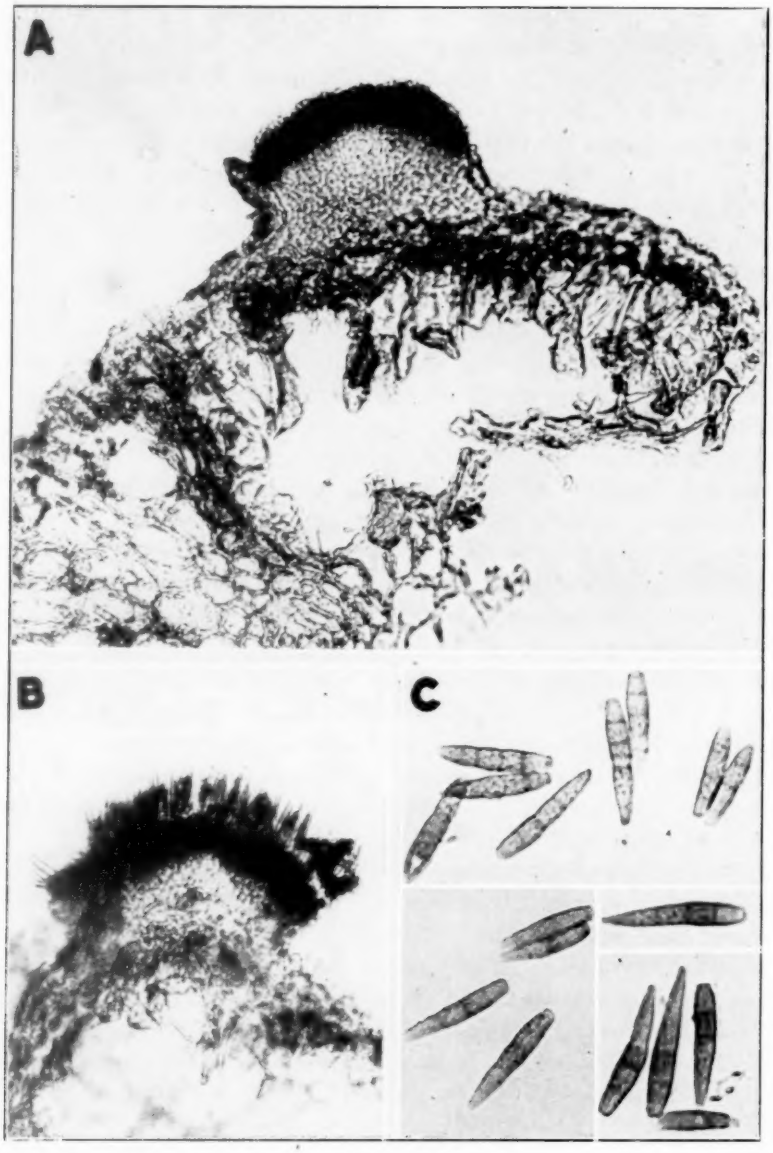

FIG. 1. *Coryneum asperulum.* A, Cross section of acervulus with spores dislodged. (Note host epidermal tissue.) ×225. B, Cross section of mature acervulus with spores attached. ×225. C, Mature spores. ×600. Photomicrographs by M. L. F. Foubert.

on leaves of *Abies pectinata* DC. in Denmark,[4] *C. thyicolum* Vest. on leaves

[4] Rostrup, E. Mykologiske meddelelser (VIII). Botanisk Tidsskrift **22**: 254–276. 1898–99.

of *Thuja occidentalis* L. in Sweden,[5] and *C. cinereum* Dearn. on older needles of *Pinus contorta* Dougl. and *P. murrayana* Grev. and Balf. in Oregon and Colorado.[6] *Coryneum asperulum* differs from *C. bicorne* by having longer and more narrow conidia. Furthermore, the conidia of *C. bicorne* are curved and bifurcate. The conidia of *C. thyicolum* are characterized by conic papillae at each end and are much broader than those of *C. asperulum*. The long conidiophores and shorter and broader conidia of *C. cinereum* differentiate it from *C. asperulum*. Karsten[7] described a fungus from the needles of *Juniperus communis* L., which he placed in the closely related genus, *Exosporium*, and stated that his species, *Exosporium deflectens* Karsten, resembled *Coryneum*. Although *E. deflectens* has 3-septate conidia, the spores are much shorter than those of *C. asperulum*.—FRANCES F. LOMBARD and ROSS W. DAVIDSON, Division of Forest Pathology, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, Beltsville, Maryland.

Reduction in Yield of the Anjou Pear Caused by Wettable-sulphur Spray.[1]—Wettable-sulphur fungicides used for control of the scab fungus (*Venturia pyrina* Aderh.) at Hood River, Oregon, were reported recently[2] to cause russeting of the fruit and lighter green color of the foliage of the Anjou pear variety. Although early observations indicated that sulphur sprays might influence the yield of fruit, completely satisfactory experimental evidence was not obtained until 1945. A summary of the effect of fungicides upon the fruit set and fruit yield of Anjou pears for 1942 through 1945 is presented in this paper.

Plots and spray program. The plots, selected primarily for pear scab control experiments, were located in 2 different orchards. In both orchards the Anjou trees were relatively vigorous and at the peak of their productivity. The trees had been pruned to prevent interlocking of limbs and excessive shading of the lower branches. Fertilization, cultural practices, and the opportunity for pollination by other varieties were considered satisfactory for commercial crop production.

All the experimental trees received a delayed dormant application of either lime-sulphur solution or lime-sulphur plus oil emulsion to control insect pests and the overwintering scab fungus in lesions on twigs. Previous evidence had indicated that the delayed dormant sprays had no adverse effect upon fruit set.

[5] Vestergren, Tycho. Bidrag till en monografi öfver Sveriges Sphaeropsideer. I. Sphaeropsideae et Melanconieae novae in Suecia collectae. Öfversigt af Kongl. Vetenskaps-Akademiens Förhandlingar **54**: 35–46. 1897.

[6] Dearness, John. New and noteworthy fungi.—III. Mycologia **16**: 143–176. 1924.

[7] Karsten, P. A. Fragmenta mycologia XXIII. Hedwigia **27**: 258–260. 1888.

[1] Published as Technical Paper No. 482 of the Oregon Agricultural Experiment Station, with the approval of the Director of the Station and the Chief of the Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture.

[2] Kienholz, J. R., and Leroy Childs. Fungicides in relation to scab and fruit russet of pear in the Hood River Valley, Oregon. Phytopath. **35**: 714–722. 1945.

The experimental spray treatments, applied from the pink through 3 or 4 cover sprays during the 4 growing seasons, were are follows: (1) Check. Lead arsenate (3–100) only. (2) Micronized wettable sulphur (8–100) plus lead arsenate (3–100). (3) Ferric dimethyldithiocarbamate (Fermate) ($1\frac{1}{2}$–100) plus lead arsenate (3–100). (4) Copper phosphate-lime-bentonite (4–4–4–100) plus lead arsenate (3–100).

All materials were applied with a commercial power sprayer which maintained a pump pressure of approximately 400 pounds. The lead arsenate (3–100) for codling moth control usually was added in the calyx and later cover sprays.

Results. Four applications (pink, calyx, and 2 later applications) were made to the plots in 1942. Treatments 1 and 3 were each applied to 6 trees; treatment 2 to 60 trees; and treatment 4 to 24 trees. The design of this experiment did not permit a statistical analysis of the results. No counts were made of the number of blossoms originally on the trees, so the percentage that set fruit could not be determined. The average yields in field boxes per tree (a field box usually holds from 100 to 150 average sized pears and

TABLE 1.—*Effects of 3 fungicides upon the set of fruit of Anjou pears*

Treatment number and fungicide used	Percentage of blossoms setting and maturing their fruits		
	1943	*1944*	*1945*
1. Check trees	0.27	3.4	2.84
2. Wettable sulphur 8–100	0.11	1.9[a]	1.80[a]
3. Fermate 1.5–100	0.17	2.5	3.04
4. Copper phosphate-lime-bentonite 4–4–4–100	0.25	3.5	3.71[a]

[a] Results differ significantly from those of corresponding check at the 5 per cent point.

weighs approximately 45 to 50 pounds) were: Check, 9.8; wettable-sulphur, 3.3; Fermate, 8.0; and copper phosphate-lime-bentonite, 10.5. The sulphur-sprayed trees yielded about one-third as much fruit as the other trees.

Three applications (pink, calyx, and first cover) were made on the test plots in 1943. An improved experimental design involving the use of 3 replicates of 2-tree plots for each treatment was used. The blossoms were counted on each tree and the percentage of fruit set determined from the number of matured pears. Spring frost injury and other unfavorable weather conditions during the pollination period caused an extremely light set of fruit. The sulphur-sprayed trees had the lowest percentage of fruit set (Table 1), but the results were not statistically significant. The actual box yields are not available.

The plots received only 3 applications (pink, calyx, and first cover) in 1944. The experimental design was modified to provide 6 replicates of single-tree plots. The blossoms were counted and the percentage of fruit set determined, as in 1943, from the number of pears on the trees at harvest time (Table 1).

The 2.5 to 3.5 per cent fruit set in treatments 1, 3, and 4 produced a rela-

tively heavy yield per tree, but the 1.9 per cent fruit set in treatment 2 (sprayed with sulphur) produced only a medium crop. The lesser fruit set in treatment 2 was statistically significant by odds of 19 : 1. The average yields, in field boxes per tree were: Check, 13.2; wettable-sulphur, 9.2; Fermate, 11.4; and copper phosphate 14.4.

Since the results in 1942, 1943, and 1944 indicated that the use of wettable-sulphur might cause serious reduction in yield, an enlarged experiment was designed in 1945. In this experiment 12 replicates of single-tree plots were distributed at random in a 16-year-old orchard. All the trees in this orchard had received uniform care for several years, and they produced a uniform and well-distributed crop in 1945, indicating that the selected site was ideal for an experiment dealing with fruit set.

Three applications (pink, calyx, and first cover) were made on all treated plots in 1945. The blossoms were counted on one main leader branch of each tree. This branch was chosen on the south or southeast side of the tree and every effort was made to select uniform limbs. The fruit counts were made after it was certain further loss of fruit would not occur.

The approximately one-third smaller set of fruit (Table 1) on the "count limbs" of trees sprayed with wettable sulphur, as compared with the trees receiving Fermate or lead arsenate only, was statistically significant. The set of fruit on the trees receiving copper phosphate was slightly greater than that of the check trees, with odds of 19 : 1 for significance. The average yields, in field boxes, by trees of comparable size in the plots were: Check, 11; wettable sulphur, 7.7; Fermate, 9; and copper phosphate, 10.9.

Since one tree may form twice as many blossoms as another of equal size, tree size does not make a true basis for a comparison of yields. The number of fruits produced from a given blossom potential provides better evidence of tree productivity.

Summary. A significant reduction in the yield of Anjou pears has been demonstrated in the Hood River Valley, Oregon, following the use of wettable-sulphur spray for pear scab control. Since wettable sulphur also has given poor scab control and caused fruit russet, it appears to be very undesirable as a fungicide for Anjou pears under conditions prevailing in the Hood River district. The appearance of fruit, yield, and foliage color have all been superior where such substitutes as copper phosphate or Fermate have been used.—J. R. KIENHOLZ, Associate Pathologist, Division of Fruit and Vegetable Crops and Diseases, and LEROY CHILDS, Superintendent, Hood River (Ore.) Branch Expt. Station.

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